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THE VALUE OF HEXAMETHYLENAMIN AS AN INTERNAL ANTISEPTIC IN OTHER FLUIDS OF THE BODY THAN URINE *

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In previous communications I have presented a method for the quantitative estimation of formaldehyd in the urine of patients receiving hexamethylenamin by mouth,[1] and the results of a study with this method of the value of the internal use of hexamethylenamin as a urinary antiseptic.[2] This study on the urine clearly indicates that the conversion of hexamethylenamin into formaldehyd is a simple chemical process which will readily occur in an acid medium, but will not occur in an alkaline medium. It also shows that the amount of this conversion is dependent on the degree of urinary acidity, the duration of exposure to the action of this acidity and the percentage concentration of the drug in it. These facts have a significant bearing on the justification of the wide-spread therapeutic use of hexamethylenamin for other than vesical conditions. It has been recommended as an antiseptic for practically all of the body fluids, the only basis for which, aside from clinical observations, is the presence of the drug in these fluids after internal administration.

Although Butlerow[3] had prepared hexamethylenamin more than thirty years before, Nicolaier,[4] in 1894, introduced it into medicine, and demonstrated formaldehyd in the urine as its decomposition product. Richardson,[5] in 1899, confirmed Nicolaier's findings. These observations immediately popularized the drug as a urinary antiseptic. In 1907, Bucura[6] found free hexamethylenamin in human milk. A year later

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* Read Jan. 5, 1914, before the Johns Hopkins Medical Society, Baltimore.

1. Hinman, Frank: Tr. Am. Assn. Gen.-Urin. Surg., May 7, 1913.
2. Hinman, Frank: An Experimental Study of the Antiseptic Value in the Urine of the Internal Use of Hexamethylenamin, Jour. Am. Med. Assn., 1913, lxi, 1601.
3. Butlerow: Ann. d. Chem. u. Pharm., 1860, cxv, 330.
4. Nicolaier: Deutsch. med. Wchnschr., 1895, xxi, 541.
5. Richardson: Jour. Exper. Med., 1899, iv, 19.
6. Bucura: Arch. f. exper. Path. u. Therap., 1907, iv, 398.

Crowe[7] showed that it is excreted in the bile, pancreatic juice and synovial and cerebrospinal fluids, and he recommended its prophylactic and curative value in diseases of the meninges of the brain and spinal cord. Barton,[8] in 1910, found hexamethylenamin in secretions of the middle ear, and since then much has been written relative to its antiseptic value in otitis media. In the same year Hanzlik[9] showed that it is excreted in saliva, and a year later Armstrong and Goodman[10] found it in the sputa of tuberculosis and of pneumonia. About the same time Gradle[11] demonstrated its presence in the anterior chamber of the eye, and in 1912 Whitman[12] found it in both the aqueous and vitreous humors. These findings have been repeatedly confirmed. All authorities are, therefore, agreed that hexamethylenamin is present after internal administration, in practically every fluid of the body.

Because of the ready conversion into formaldehyd in the urine, it has been assumed by some investigators that it acts in a similar manner in these other fluids, while other observers have presumably regarded hexamethylenamin itself as of antiseptic value. In the literature there is no evidence of free formaldehyd having been demonstrated definitely in any of these body-fluids (urine excepted). Hanzlik[9] was unable to find it in the saliva; Schmid and Schroeter[13] failed to find it in human milk; Burnam[14] could not demonstrate it in the cerebrospinal fluid or bile, and more recently Hanzlik and Collins[15] have stated positively that free formaldehyd is never present in saliva, milk, bile, cerebrospinal, synovial, pericardial and pleural fluids of man or in the vitreous or aqueous humors of animals. The apparent confusion on this point is due to the character of tests employed. In those cases in which free formaldehyd has been reported as present in these fluids after the ingestion of hexamethylenamin, the tests that have been used employ strong acids, which, by their action on the hexamethylenamin, liberate formaldehyd, which then reacts.

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7. Crow, J. S.: Johns Hopkins Hosp. Bull., 1908, xix, 109; ibid., xx, 102.
8. Barton, W. M.: The Elimination of Hexamethylenamin by the Mucous Membrane of the Middle Ear, Jour. Am. Med. Assn., 1910, liv, 871.
9. Hanzlik, P. J.: The Excretion of Hexamethylenamin in the Saliva, Jour. Am. Med. Assn., 1910, liv, 1940.
10. Armstrong, J. J., and Goodman, E. H.: The Excretion of Salicylic Acid and Hexamethylenamin in the Sputum, Jour. Am. Med. Assn., 1911, lvi, 1553.
11. Gradle, W. S.: Ophth. Rec., March, 1911.
12. Whitman: Arch. Ophthalmol., 1912, xli, 604.
13. Schmidt and Schroeter: Centralbl. f. Physiol. u. Path. d. Stoffwechsel, 1910, v, 129.
14. Burnam, C. F.: An Experimental Investigation of the Value of Hexamethylenamin and Allied Compounds, THE ARCHIVES INT. MED., 1912, 324.
15. Hanzlik, P. J., and Collins, R. J.: Hexamethylenamin: The Liberation of Formaldehyd and the Antiseptic Efficiency under Different Chemical and Biological Conditions, THE ARCHIVES INT. MED., 1913, xii, 578.

As an example of this may be cited the results of Ibrahim,[16] who in 1910 presented a bacteriological study of the cerebrospinal fluid with reference to the inhibitory action of hexamethylenamin on different organisms (*Staphylococcus aureus, Bacillus coli, B. typhi abdominalis* and *B. pyocyaneus*) with these conclusions:

1. After the internal administration of hexamethylenamin this can be demonstrated in the cerebrospinal fluid of infants.

2. Hexamethylenamin in solution in cerebrospinal fluid forms, on standing at 37 C., formaldehyd.

3. Hexamethylenamin gives in the cerebrospinal fluid at body temperature bactericidal action.

4. On the grounds of this activity it appears justified to administer hexamethylenamin as a therapeutic measure in all forms of serous and purulent meningitis.

Ibrahim did not recognize the significance of acidity in the liberation of formaldehyd. He used three tests: Nicolaier's[17] *Bromwasserprobe*, Jorrison's test with the use of acetic acid,[18] and Hehner's test (sulphuric acid). The first is a test for hexamethylenamin and the other two, because of the acid used, are unreliable. With regard to the antiseptic findings the bacterial counts are not very convincing, and I was unable to confirm them in three cases in which, after the addition of about 0.05 gm. hexamethylenamin to 5 c.c. of spinal fluid, there was no inhibition of either *Staphylococcus aureus* or *Bacillus coli* after seventy-two hours' incubation, as shown by the count after transferring one oese to an agar-plate, although the delicate tests (phloroglucin and amidol) for formaldehyd were present.

The importance of acidity to the decomposition of hexamethylenamin has but just recently been recognized. Wiggs[19] suggested its influence in the urine in 1910. Jordan,[20] a year later, reported that formaldehyd was not found in alkaline urines, and recently studies by Smith,[21] Hanzlik and Collins[15] and myself[2] have independently demonstrated the importance of the degree of urinary acidity in the use of hexamethylenamin as a urinary antiseptic. The excellent paper of Hanzlik and Collins appeared not long ago, and, since it is in accord largely with the present study, a few of their conclusions will be given, and are as follows:

Alkalies prevent, while acids facilitate the liberation of formaldehyd from hexamethylenamin in all body fluids.

16. Ibrahim: Med. Klin., 1910, p. 48.
17. Nicolaier: Ztschr. f. klin. Med., 1899, xxxviii, 350.
18. Jorrison: Chem. Ztz., 1897, 290.
19. Wiggs: South. Med. Jour., December, 1910.
20. Jordan: Biochem. Jour., 1911, v, 274.
21. Smith, G. G.: Bost. Med. and Surg. Jour., May 15, 1913.

The liberation of formaldehyd from hexamethylenamin in pathological fluids obeys the same laws as in other solutions, that is, it can only occur in acid reaction.

After administration, hexamethylenamin is present, but does not liberate free formaldehyd in blood, cerebrospinal, pleural, pericardial and synovial fluids, vitreous and aqueous humors, and urine when truly alkaline.

The beneficial therapeutic effects of hexamethylenamin depend on the liberated formaldehyd. Such effects are to be expected principally, if not always, in acid urine only.

The present work embodies the testing for formaldehyd of both normal and pathologic fluids of patients being fed hexamethylenamin with a study of the action of these fluids *in vitro* toward the liberation of formaldehyd when subjected to infection. The main question at issue was to determine whether infection could alter the reaction of these normally alkaline fluids sufficiently to produce the liberation of formaldehyd, and, if so, whether there would be enough liberated to produce antisepsis.

ANTISEPTIC VALUES OF FORMALDEHYD AND HEXAMETHYLENAMIN

A review of the work of other investigators and my own experiments[1] indicates that formaldehyd is a slow and a relatively weak germicide, but that, up to certain dilutions, it exerts a powerful inhibitory influence on bacterial development. This power of inhibition varies slightly with different organisms. A dilution of liquor formaldehydi of about 1:16,000 will cause complete inhibition of the growth of *Bacillus typhosus* for twenty-four hours; a dilution of about 1:6,000 will completely kill the organism in that time; about 1:30,000 dilution will partially restrain its growth, while a 1:40,000 dilution has no apparent effect unless the results are shown by means of plating and a systematic bacterial count when a slight diminution in the count is obtained. Higher dilutions have no inhibitory effect.

Hexamethylenamin has been shown repeatedly (Burnam, Jordan, Hanzlik and Collins) to have no germicidal or antiseptic power. Hanzlik and Collins found that hexamethylenamin, to which 0.5 per cent. ammonium hydroxid had been added to prevent the liberation of formaldehyd, in as strong a dilution as 1:50 did not inhibit the growth of the organisms studied. In my experiments bacteria (*B. typhosus, B. coli, M. pyogenes aureus*) grew as readily in a slightly alkaline broth to which hexamethylenamin had been added in the proportion of 1:100 as in normal broth.

It is seen, therefore, that hexamethylenamin is of value as an antiseptic only after its conversion into formaldehyd, which must be present in the proportion, at least, of about 1:30,000.

TESTS EMPLOYED FOR FORMALDEHYD AND HEXAMETHYLENAMIN

Four different tests were made use of in the examination of material. Hehner's test[22] was used to show the presence of hexamethylenamin. It is really a test for formaldehyd, but the strong sulphuric acid used in the test quickly converts the hexamethylenamin into formaldehyd, which then reacts, so that it is given equally well by both drugs. The amidol[23] and Jorrison's[18] phloroglucin tests were used to detect small amounts of formaldehyd not shown by the Rimini test. The amidol test, although very delicate, is not particularly reliable, and is not applicable to all fluids. The phloroglucin test is both delicate and reliable. Neither test is given by hexamethylenamin. Both are qualitative tests only. Rimini's[24] test was modified so as to be used for quantitative estimation[1] of formaldehyd, for which it is reliable up to dilutions of about 1:60,000. It is not given by hexamethylenamin. If formaldehyd is present in less amount than 1:60,000, it can be of no practical importance inasmuch as it would not be antiseptic; but the amidol and phloroglucin tests would show even minute quantities (up to 1:10,000,000). With these tests it is possible to determine the presence or absence of both hexamethylenamin and of formaldehyd, to determine whether hexamethylenamin is present alone and if formaldehyd is present, to estimate the quantity up to 1: 60,000.

EXAMINATION OF NORMAL AND PATHOLOGIC FLUIDS

The material for examination consists of that from eleven cases of cerebrospinal and ventricular fluids, four cases of otitis media, two cases of empyema, one case of pelvic abscess, six normal blood-serums, two cases from which normal bile was obtained, three from which sweat and five from which saliva was collected. This material may seem small in amount from which to draw conclusions, but the main object in view was experimental and not clinical. Each fluid was subjected to many different tests with particular reference to changes in reaction and the influence of these changes on the liberation of formaldehyd. With two exceptions all patients had been given hexamethylenamin by mouth before the fluids were collected. In no case was free formaldehyd found. All showed the presence of hexamethylenamin. After the conversion of this hexamethylenamin with acid there was in no case enough formaldehyd liberated to furnish antisepsis. These findings will be outlined briefly and tabulated.

Cerebrospinal Fluid.—The spinal or ventricular fluid was obtained by lumbar and ventricular puncture in several cases and tested as shown

22. Hehner: Abstr., Lond. Chem. Soc., 1896, A, ii, 583.
23. Referred to in Allen: Commercial Organic Chemistry.
24. Rimini: Chem. Zentralbl., 1898, i, 1152; abstr., Jour. Soc. Chem. Industr., 1898, 697.

TABLE 1.—EXAMINATION OF CEREBROSPINAL FLUID

Case	Diagnosis	Material and Treatment	Tests*			
			Date	Hehner	Rimini	Amidol or Phloroglucin
1	Tuberculous meningitis.	Hexam. 15 gr., t. i. d., p. c., for several days. Lumbar puncture, 11 c.c. fluid	4/24	+	—	—
		Crystals of acid sodium phosphate added to 3 c.c.	4/24			
			4/26	+	—	+
		5 c.c. inoculated with *M. pyo. aur.*	4/24			
		Incubated at 37 C. for six days	4/30	+	—	+
2	Tuberculous meningitis.	Hexam. 20 gr., t. i. d., p. c. Lumbar puncture	4/26	+	—	—
3	Lues.	Hexam. 20 gr., q. 2 h., two days. Lumbar puncture		+	—	—
		Crystals of Na_2HPO_4 added to 5 c.c. after twenty-four hours		+	†	+
4	Medical.	Hexam. 20 gr., q. h., added 4 doses. Lumbar puncture		+	—	—
		4 c.c. inoculated with *B. coli* after forty-eight hours		+	—	+
5	Medical.	Hexam. 20 gr., q. h., added 4. Lumbar puncture		+	—	—
6	Hydrocephalus in child.	Hexam. 5 gr., q. h., added 8. Ventricular puncture		+	—	—
		5 c.c. inoculated with broth culture of meningococcus after fourteen days' incubation		+	—	+
7	Medical.	No hexam. by mouth. Lumbar puncture		—	—	..
		Hexam. 1 gr. added to 3 c.c. and tested after standing forty-eight hours		+	—	—
		Crystals of Na_2HPO_4 added to 3 c.c. with hexam. 1 gr. and tested after twenty-four hours		+	‡	..
8	Hydrocephalus in child.	Hexam. 40 gr., q. d., for three days. Lumbar puncture		+	—	—
		Ventricular puncture		+	—	..
		Above after standing three days		+	—	..
9	Hydrocephalus	Hexam. 5 gr., t. i. d., p. c., for two weeks. Lumbar puncture		+	—	..
		Ventricular puncture		+	—	..
10	Hydrocephalus in child.	Hexam. 5 gr., t. i. d. Ventricular puncture		—	—	—
		8 c.c. made strongly acid with H_2SO_4 after twenty-four hours		—	—	..
11	Meningitis.	Hexam. 40 gr., in day; 75 gr. after midnight. Lumbar puncture at 10 a. m.		+	—	—
		5 c.c. inoculated with *M. pyo. aur.* incubated five days		+	†	..

* In this and the following tables, + = positive, —, negative.
† 1:60,000. ‡ 1:20,000.

in Table 1. Hexamethylenamin was present in all of the cerebrospinal fluids with the one exception. In Case 10 the fluid obtained by lumbar puncture was negative to Hehner's test, and the addition of sulphuric acid failed to render the test positive for formaldehyd. In none of these fluids in which hexamethylenamin was present following the administration of the drug by mouth was free formaldehyd found. After inoculation of these fluids containing hexamethylenamin with the meningococcus, *M. pyogenes aureus* or *B. coli*, formaldehyd was not liberated in amounts sufficient to give the Rimini test (1:60,000), although after several days' incubation they were all positive to the delicate amidol or phloroglucin tests for formaldehyd. The addition of acid sodium phosphate to the fluid in one case (Case 3) in which the patient had received enormous doses of hexamethylenamin, 20 grains every two hours, caused after twenty-four hours the liberation of formaldehyd in about 1:60,000 dilution. Hexamethylenamin added directly to these fresh fluids was not

TABLE 2.—EXAMINATION OF PURULENT DISCHARGES IN OTITIS MEDIA

Case	Material and Treatment	Tests		
		Hehner	Rimini	Amidol or Phloroglucin
1	Hexam., gr. 5, q. 2 h., added doses 6. Discharge obtained by syringing out the ear with small amount of sterile water	—	—	—
2	As above	—	—	—
3	As above	—	—	—
4	As above	—	+	—
5	As above	—	—(?)	—

decomposed, and the subsequent inoculation of these concentrated solutions in no case caused a liberation of formaldehyd in a greater amount than 1:60,000.

Purulent Discharge in Otitis Media.—The material was obtained in cases of acute otitis media by syringing out the ear with as small an amount of sterile water as possible. With the exception of one case, which gave a positive Hehner's test for hexamethylenamin, all failed to show either hexamethylenamin or formaldehyd. The findings are not accurate, inasmuch as the discharges were necessarily diluted before being tested, but the failure to find even minute quantities of either drug are significant. The findings are grouped in Table 2.

Pus of Empyema.—Two patients with empyema were given hexamethylenamin for a few days, and at operation the pus was obtained and examined as is shown in Table 3. All tests were uniformly negative,

showing the absence of both hexamethylenamin and formaldehyd; but the pus was strongly acid in reaction, and when hexamethylenamin was added to it *in vitro* it was quickly decomposed into formaldehyd.

Pelvic Abscess and Pus-Tube.—One patient was given hexamethylenamin, 20 grains, after each meal for two days before an operation for pus-tubes and a pelvic abscess. The pus obtained at operation was examined with negative results for all tests. The reaction of the pus was strongly acid, 9.7 c.c. tenth-normal sodium hydroxid to 10 c.c., and when examined four hours after the addition of 5 grains of hexamethylenamin to 40 c.c. of it, formaldehyd was present in the proportion of 1:10,000.

Blood-Serum.—Venous puncture was done in six cases in which the patients were being given a routine dosage of 15 grains of hexamethylenamin after each meal, and the serum after separation was tested with negative results in all cases for formaldehyd, but positive in all for hexamethylenamin. The addition of acid sodium phosphate to these serums in no case caused a liberation of formaldehyd in sufficient amount to give a 1:60,000 test, although the more delicate formaldehyd tests were uniformly present after this treatment.

TABLE 3.—EXAMINATION OF PUS OF EMPYEMA

Case	Material and Treatment	Reaction	Test		
			Hehner	Rimini	Amidol or Phloroglucin
1	Hexam., 10 gr., t. i. d., p. c. Operation after two days and 600 c.c. of pus obtained which showed a pure culture of pneumococcus.....	13.6 c.c. Tenth-normal sodium hydroxid to 10 c.c.	—	—	—
	Hexam. added to 200 c.c. of above examined after twenty-four hours.....	.....	+	*	+
	(Culture made from this on the seventh day was negative) after seven days.....	After 7 days.	+	†	..
2	Hexam., 15 gr., t. i. d., p. c. Operation three days, 300 c.c. pus obtained.....	1.6 c.c.	—	—	—

* 1:10,000. † 1:7,000.

Sweat.—Two patients receiving hexamethylenamin, 15 grains, after each meal, were placed in a sweat-cabinet, their perspiration collected[25] and examined with positive results in both cases for hexamethylenamin. The Rimini test was negative in both. Unfortunately, the more delicate

25. Through the kindness of Dr. Harry Plaggemeyer.

phloroglucin test was not tried in these cases. Sweat is slightly acid in reaction, and possibly the small amount of hexamethylenamin present had been converted into formaldehyd, which would also give the delicate Hehner's test. It was not, however, present in anything like an antiseptic strength.

Saliva.—The saliva was collected from five patients receiving a routine dosage of 15 grains after each meal. A positive Hehner's test was obtained in all but one case, but the other tests were all uniformly negative.

Bile.—Hexamethylenamin when taken by mouth is excreted in the bile. Free formaldehyd, however, is not found. After the addition of hexamethylenamin to sterile bile *in vitro* even in the proportion of 1 : 100, it is not decomposed so long as the bile is kept sterile. Infection of these concentration bile solutions causes a liberation of formaldehyd, but in the two cases tested not more than 1 : 60,000 was found. There has been no opportunity to examine the contents of the gall-bladder in cases of infection after feeding hexamethylenamin. It is possible that in cholecystitis or empyema of the gall-bladder its contents may have a sufficient acidity readily to convert the hexamethylenamin excreted into it.

Hexamethylenamin in Standard Broth.—In order further to test the influence of infection on the reaction of fluids and on the subsequent liberation of formaldehyd, known dilutions of hexamethylenamin were made up in standard broth, inoculated with various organisms and tested after certain periods for changes in reaction and for free formaldehyd. The findings are given in Table 4. The changes in reaction are slight in all cases, so much so that in no case was more than 1 : 60,000 formaldehyd found after the growth of an organism in a 1 : 1,000 hexamethylenamin solution for as long as five days, and the growth of the organism was in no case inhibited.

SUMMARY OF EXAMINATION OF NORMAL AND PATHOLOGIC FLUIDS

The amount of decomposition of hexamethylenamin into formaldehyd is so clearly dependent on acidity and concentration that the problem resolves itself into a determination of these factors. None of these fluids are normally acid, and whatever tendency there is for them to become acid is being continually counteracted by the alkaline circulation. On the other hand, the amount of hexamethylenamin present in these fluids has been found to be relatively small, which is no doubt due to the fact that it is rapidly eliminated by the kidneys and is not given an opportunity to accumulate. Formaldehyd, if it is liberated within the body, is

TABLE 4.—Tests of Hexamethylenamin in Standard Broth

Organism	Dilution	24 Hours			48 Hours			72 Hours			120 Hours		
		Reaction*	Formaldehyd†		Reaction*	Formaldehyd		Reaction*	Formaldehyd		Reaction*	Formaldehyd	
			Rimini	Amidol		Rimini	Amidol		Rimini	Amidol		Rimini	Amidol
B. coli	1:1,000	Alk. 1	—	+	Acid 2	—	+	Acid 4	‡	+	Acid 4	‡	+
	1:10,000	Alk. 1	—	+	Acid 4	—	+	Acid 6	—	+	Acid 6	—	+
B. typhosus	1:1,000	Acid 4	‡	+	Acid 6	‡	+	Acid 6	‡	+	Acid 6	‡	+
	1:10,000	Acid 2	—	+	Acid 4	—	+	Acid 6	—	+	Acid 6	—	+
B. proteus vulgaris	1:1,000	Acid 1	—	+	Acid 4	—	+	Acid 4	‡	+	Acid 6	‡	+
	1:10,000	Acid 2	—	+	Acid 4	—	+	Acid 6	—	+	Acid 6	—	+
Streptococcus pyogenes aureus	1:1,000	Alk	—	—	Alk.	—	—	Acid 2	—	+	Acid 8	‡	+
	1:10,000	.....	..	..	.....	..	..	.....	..	..	.....	..	.
Meningococcus.	1:1,000	Alk.	—	—	Acid 2	—	+	Acid 4	—	+	Acid 4	—	+
Alkaligenes	1:1,000	Alk.	—	—	Alk.	—	—	Alk.	—	—	Alk.	—	—
Control typhoid	0	Alk.	—	—	Acid 2	—	—	Acid 4	—	—	Acid 8	—	—
Contral hexamethylenamin	1:1,000	Alk.	—	—	Alk.	—	—	Alk.	—	—	Alk.	—	—
Control urine	1:1,000	Acid	¶	+	Acid 2.6 §	**	+	Acid 2.6 §	**	+			

* The numerals under "Alk." or "Acid" indicate the number of drops of tenth-normal hydrochloric acid (alk.) or tenth-normal sodium hydroxid (acid) necessary, respectively, to neutralize 10 c.c. of the solution with phenolphthalein as indicator.

† In the tests, + means positive and — negative.

‡ Percentage of formaldehyd, 1:60,000.

§ The figures in the lowest column under "Acid" indicate the number of cubic centimeters of tenth-normal sodium hydroxid needed to neutralize 10 c.c. of urine.

an irritant, and as such is not tolerated by the organism. Maguire[26] found that intravenous injection of formaldehyd into animals produced gastric irritation with ulcer formation, hematuria and hemoglobinuria and death. Several of my patients were fed liquor formaldehydi (in dilution of 1:3,000, 6 ounces every three hours), and afterward their blood and urine were repeatedly found to be negative to all tests for both hexamethylenamin and formaldehyd, showing that the body can in some way burn up these small amounts. The fate of the formaldehyd which gets into the circulation is uncertain. There is experimental evidence to suggest that when not excreted by the kidneys it is neutralized into a harmless polymer and possibly hydrolyzed in the liver into one of the sugars. The conditions, therefore, for the decomposition of hexamethylenamin in antiseptic amounts are seldom, if ever, realized in these alkaline body-fluids.

From these facts and from the uniform negative findings on direct examination it is evident that hexamethylenamin can be of no value in the alkaline or neutral fluids of the body, such as found on the serous surfaces in general. This will be true even when enormous doses of the drug are administered, which is shown by no conversion occurring in these fluids when large amounts of the drug are added directly to them. Hexamethylenamin, therefore, can be of no prophylactic benefit in any of the body-fluids except those that are normally acid, and the only three fluids of this character are the urine, sweat and gastric juice.

The question of the influence of infection on these alkaline fluids relative to the liberation of formaldehyd is more difficult to answer. The examinations for formaldehyd of the pathologic fluids of patients given hexamethylenamin by mouth were all uniformly negative. After the subsequent addition of hexamethylenamin to these fluids, with the exception of localized accumulations of pus, no decomposition of this into formaldehyd was found. In local accumulations of pus, as in empyema or abscesses, this may have a pronounced acidity, and the addition of hexamethylenamin even in amounts of 1:10,000, to this pus quickly liberates formaldehyd. *In vivo,* however, these pus-pockets are apparently so well walled off that very little of the hexamethylenamin in the blood finds its way into them. If hexamethylenamin did enter in at these local areas, the formaldehyd formed there would naturally have as ready access to the circulation and would be carried off as fast as formed, so that no accumulation for antiseptic purposes would occur. The gall-bladder, in those infections in which its contents become acid, may be a possible exception.

The subsequent inoculation with organisms of fluids obtained from patients taking hexamethylenamin by mouth, even on seven days' incu-

26. Maguire: Med. News, 1903, lxxxiii, 393.

† Percentage of formaldehyd, 1:60,000.
‡ The figures in the lowest column under "Acid" indicate the number of cubic centimeters of tenth-normal sodium hydroxid needed to neutralize 10 c.c. of urine.

bation, in no case liberated formaldehyd in antiseptic amounts, although the more delicate tests of those showing formaldehyd up to 1 : 10,000,000 were sometimes obtained. And after the addition of as much as 1 : 1,000 hexamethylenamin to these fluids and then their inoculation, formaldehyd was liberated only after several days in 1 : 60,000 amounts. The inoculation of broth solutions of hexamethylenamin in no case showed greater conversion than 1 : 60,000, and then only after seventy-two hours. Infection of these fluids *in vitro*, therefore, even under the most favorable circumstances, that is, when hexamethylenamin has been added in as high a dilution as 1 : 1,000, a concentration that would never obtain *in vivo*, does not produce sufficient change in reaction to form formaldehyd in antiseptic strength. Furthermore, those fluids containing hexamethylenamin after its ingestion by mouth, when made strongly acid with acid sodium phosphate or sulphuric acid, did not contain hexamethylenamin in amounts to liberate more than 1 : 60,000 formaldehyd, showing that hexamethylenamin was not present in sufficient concentration to furnish antisepsis even should all present be decomposed.

It is reasonable to assume, therefore, that infection in the body does not change the reaction of the body-fluids into which hexamethylenamin is secreted sufficiently to cause its conversion in antiseptic amounts. In case of localized accumulations of pus of marked acidity, hexamethylenamin does not gain access in amounts to be of value, and in the case of other infection in communication with the circulation, the small amount of formaldehyd formed is quickly taken up and metabolized or excreted by the kidneys.

CONCLUSIONS

Hexamethylenamin is dependent on the liberation of formaldehyd for its antiseptic value.

Hexamethylenamin is not converted into formaldehyd in any of the normal alkaline fluids of the body; therefore, it can be of no prophylactic value in any of these fluids.

After some infections of these fluids there may be under certain conditions a change in reaction sufficient to produce slight liberation of formaldehyd, but it was not possible to show that there would be enough to give antisepsis.

In localized infections of pronounced acidity, hexamethylenamin is not taken into them from the circulation in amounts to furnish formaldehyd in antiseptic strength (the gall-bladder, possibly, excepted).

The therapeutic use of hexamethylenamin as an internal antiseptic is justified, experimentally, for urinary conditions alone, and then only when it is excreted into an acid urine.

HEXAMETHYLENAMIN *

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Hexamethylenamin is widely used as an antiseptic to prevent and to combat various infections of the serous cavities and blood as well as those of the urinary tract, the wide range of application being due to the striking effects obtained in some cases of cystitis and allied conditions, as well as to the great penetrability of the drug, which after ingestion can be found in every fluid of the body.[1] These facts, as well as the possible liberation of free formaldehyd, to which the efficiency of the drug is usually ascribed, would seem to make it an ideal drug in infections, and great claims have been made for its efficacy. But the question arises: Is formaldehyd liberated, and if so, is it in sufficient amount to have an antiseptic action?

Hexamethylenamin is readily decomposed into formaldehyd and ammonia in acid solutions, less readily in neutral or slightly alkaline solutions, and not at all in a stronger alkaline medium. When injected intravenously it produces a temporary increase in the rate and depth of respiration which would indicate an ammonium action and a consequent formaldehyd liberation. In addition, formaldehyd is always found in acid urines after administration of the drug. These results might mean a liberation of formaldehyd in all parts of the body; but Hanzlik and Collins[2] and others have failed to find such a liberation in any of the body fluids except the gastric juice and urine. There is a possibility, however, that it is liberated and yet not detected, since one of us (McGuigan) has shown that small amounts of formaldehyd are oxidized in the blood so rapidly that it quickly escapes detection by the ordinary means, even that introduced into the stomach or liberated from hexamethylenamin by the gastric juice and subsequently absorbed. To meet this objection a new method is offered for the detection of free formaldehyd in the blood. This consists in dialyzing the normal circulating blood in animals through collodion tubes interposed between the carotid

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* From the Laboratory of Pharmacology of the Northwestern University Medical School.

* Submitted for publication Dec. 29, 1913.

1. McGuigan, Hugh: Jour. Biol. Chem., 1912, xi, 33, proceedings.

2. Hanzlik, Paul J., and Collins, R. J.: Hexamethylenamin: The Liberation of Formaldehyd and the Antiseptic Efficiency Under Different Chemical and Biological Conditions, THE ARCHIVES INT. MED., 1913, xii, 578. This gives a complete review of the literature.

artery and the external jugular vein.[3] The tubes are immersed in water or saline solution and the living blood is dialyzed during the action of the drug. If any formaldehyd is formed, part of it will rapidly pass into the dialysate, where it can be identified by the usual tests. The results show uniformly that hexamethylenamin is found in the dialysate in a few minutes after the injection, while free formaldehyd is not detected even after several hours of dialysis. The following protocol will illustrate:

Male dog. Ten kg. Ether anesthesia.

1: Collodion dialyzing tubes attached to the carotid artery and external jugular vein.

1:20: Dialysis commenced. Tests of the dialysate for hexamethylenamin and formaldehyd negative. 100 c.c. of 3 per cent. hexamethylenamin in 0.9 sodium chlorid slowly injected into the femoral vein in the next ten minutes.

1:40: Dialysate tests: Hexamethylenamin positive; formaldehyd negative.

2: Dialysate tests: Hexamethylenamin positive; formaldehyd negative.

4:10: Dialysate tests: Hexamethylenamin positive; formaldehyd negative. Urine strongly positive to free formaldehyd tests.

4:30: During the next ten minutes 40 c.c. 0.5 per cent. formaldehyd injected slowly into the femoral vein.

4:40: Dialysate test shows free formaldehyd.

In control experiments the injection of formaldehyd only always gave free formaldehyd in the dialysate. Some hexamethylenamin is also formed. The Jorrisen and Rimini tests were used for free formaldehyd, and Hehner's test for the combined form.

It is evident that formaldehyd is not liberated in amounts sufficient for detection with the phloroglucin or phenylhydrazin tests. Since these reagents will detect amounts far less than enough to have any antiseptic action, hexamethylenamin antisepsis, if any, cannot be due to decomposition products. But even though formaldehyd were liberated in minute amounts it would not have the reputed hexamethylenamin effect, since after injection small amounts of free formaldehyd are so rapidly oxidized that antiseptic action is improbable, while in larger amounts it is decidedly injurious.

Hexamethylenamin itself has no antiseptic action on yeast or bacteria outside of the body, but the possibility still remains that the mere entrance and exit of the drug may have a beneficial action when taken internally.

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3. Abel, John J., Rowntree, Leonard G., and Turner, B. B.: Removal of Diffusible Substances from the Circulating Blood of Living Animals by Dialysis, Jour. Pharmacol. and Exper. Therap., 1914, v, 275. This gives Abel's method without a coagulant. We were unaware of the priority of Abel in this field when the work was done.

SUMMARY

Since, after the administration of hexamethylenamin, free formaldehyd appears only in the gastric juice and acid urine, and not in other secretions or in the blood, even with dialyzing tests, the liberation results from the acid reaction only and not from cell action. Even if it were liberated in other locations it would probably exert no beneficial action because of its reconversion or rapid oxidation into injurious products. Combined formaldehyd as hexamethylenamin, does not decompose readily until an acid medium is reached, when the action is that of weak formaldehyd. The only apparent indication for the internal administration of the drug, therefore, is in infections of the urinary tract.

SOME RELATIONS OF THE BRAIN AND OF THE OLFACTORY APPARATUS TO THE PROCESSES OF IMMUNITY *

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The body is provided with three obvious lines of defense against infection, namely, its peripheral epithelium, its blood and lymph, and its tissue cells. The activity of modern investigators has been engrossed almost wholly with study of the last two; the first has received very little attention. Yet it is indisputable that the epithelial covering of the body must have intrinsic resources of protection against adverse influences which are in such habitual action that its relations should be considered truly physiological, rather than pathological, as its response to adventitious agencies would be. In short, a study of the pathologic physiology of the peripheral epithelium must conceive of it primarily as a mechanism for the *prevention* of disease rather than, as in the case of the body-fluids and tissues, an establishment for producing the means of *cure*. While the protoplasm of the surface cells must be endowed with a degree of autonomy involving capacity for independent response to irritation, which possibly includes the production of specific antibodies, its striking peculiarity is its abundant supply of sensory nerves, largely endowed with "specific energy," through which every external stimulus is made to provoke an immediate and universal internal response.

The incitement to the investigation the report of which follows was a desire to develop the existence of some immunological relations between the peripheral and the central mechanisms. If *a priori* conceptions are given undue prominence at the outset, a measure of defense may be found in the wise dictum of Richet,[1] that in biologic research the guiding thread must always start from the teleologic point of view.

Modern therapeutics offers abundant illustration of the beneficent influence of physical agencies acting externally. The criterion for the usefulness of cold water in the treatment of typhoid fever is the "physiological response" which its application may provoke. As Chantemesse points out, the hydriatric stimulus probably does not excite the formation of specific antibodies against the invading noxa, but it seems probable that the *capacity* for the formation of antibodies is enhanced indi-

* Submitted for publication Dec. 17, 1913.

1. Richet, Charles: Ann. de l'Inst. Pasteur, 1907, xxi, 497.

rectly, through reflex excitement of the various motor and metabolic mechanisms. Similarly, the benefits of the open-air treatment in tuberculosis are in some way due to physiological response to external agents, which, as in another place I have attempted to show, exert their influence largely through the temperature nerves of the skin. There is good reason to believe that at least the vital capacity for the formation of antibodies is largely under the control of external stimuli which make up the environment of what is known as the "hygienic life." The fact that tuberculosis finds least resistance to its development in the tissues of those who, like the American Indians, from a habitual open-air life are suddenly forced to conform to the restraints of civilization, argues that hygienic living produces no specific antibodies against this disease.

But there is abundant clinical evidence that a great variety of antigens can produce specific anaphylactic systemic response when applied to the peripheral epithelium. The assumption that such antigens must be absorbed into the circulation before becoming effective, while probably correct in general, is unwarranted without direct evidence. The urticarias and other anaphylactic phenomena following the ingestion of certain articles of food by some people, and, especially, the asthmatic paroxysms induced by the inhalation of certain odors, belong to one of the most interesting divisions of clinical pathology. Indeed, it was an incident related to me by V. C. Vaughan, Jr., of Detroit, which gave the final impulse to the present investigation.

Vaughan's account is as follows: The patient was a man, aged 36, who was suffering from moderately advanced tuberculous disease of the lungs complicated by recurrent hemoptyses. From March 11 to 13, inclusive, the patient received three injections of fresh, normal rabbit's serum of 0.1 c.c., 9 c.c. and 10 c.c. April 7, twenty-seven days after the first injection, a rabbit was brought into the patient's room, was handled by him and allowed to run about the bed. Between three and five hours after this event the patient suffered an asthma-like attack — the first which he had ever experienced.

As indicated at the outset, my conception in the main has been that phenomena which are essentially anaphylactic form a conspicuous feature of normal sensory activity. The mysterious ability of the hound to follow a definite trail unconfused by a multitude of other footsteps is cleared up at once if we assume that the dog's olfactory apparatus is sensitized to the odor which he follows. One is tempted to suggest that even the phenomena of complementary color sensation, as manifested in the "after-images," are but the psychic signs of specific antibodies formed in the retina.

The experiments to be described were performed on guinea-pigs. Sensitization was achieved by subcutaneous injection of normal horse-

serum obtained from a commercial source. The sensitizing dose usually varied from 0.25 to 0.5 c.c. The toxic injection was invariably given through the jugular vein. Many intracranial operations were performed, including cutting the olfactory nerves or ablation of the olfactory lobes alone or with a certain bulk of the anterior cerebrum. Anatomically the guinea-pig is excellently adapted for such work. As is well known, the anatomical relations of the sense-organ for smell are almost unique.[2] It is constructed peripherally of nerve-cells inclosed within the olfactory mucous membrane. Processes directed distally from the nerve-cells project freely like bristles from the free surface of the membrane, while from the opposite poles "teledendrons" find their way into the cranial cavity to embrace the nerve-cells of the olfactory lobes.

This research would have been quite impossible but for the devoted aid given by my colleague, Dr. Cuthbert Powell, who has freely lent his surgical skill to the performance of the many scores of surgical operations involved in the investigation.

GUINEA-PIGS SENSITIZED TO HORSE-SERUM MAY BE DESENSITIZED BY SOJOURN IN A HORSE STABLE

The first effort made was to elicit some sign of anaphylaxis in the peripheral epithelium of a serum-sensitized animal. A guinea-pig, which had received a subcutaneous injection of horse-serum thirty-eight days before, was held gently on its back and horse-serum in droplets was allowed to fall into each nostril. I expected to witness some sign of specific irritation. The result, however, was wholly negative. The animal a few hours later received an intravenous injection of 0.5 c.c. of horse-serum and died in about three minutes with characteristic symptoms. Another sensitized pig was made to inhale finely vaporized serum, in the manner to be described later, but, as in the first case, the results were negative. These observations were repeated on numerous occasions with different animals and the results all pointed to the conclusion that *guinea-pigs sensitized by subcutaneous injections of horse-serum manifest no particular irritation when the same serum is instilled into the nose or given by inhalation.*

It then occurred to me that possibly the aroma of the animal producing the sensitizing antigen might develop some characteristic reaction. Accordingly, four guinea-pigs were placed in a box covered with wire netting. Two of the animals were normal and two, forty days previously, had each received subcutaneously from 0.5 to 0.75 c.c. of serum. The box was carried, July 7, to a livery stable in which a score or more of horses were maintained. The air of the stable at this time was powerfully impregnated with a characteristic odor and there

2. Troland: Am. Jour. Physiol., 1913, xxxii, 19.

was a particularly pungent quality to the smell. Later in the season, as cooler weather advanced, the intensity of the odor diminished and its pungent quality disappeared. There was, as will be seen directly, a coincident change in the biological reactions. The box had been in the stable possibly five minutes when marked nervous disturbances were manifested by the two sensitized animals. One of them, a female weighing 480 gm., showed marked excitement, dashing about the box and biting at her companions, then scratching the nose vigorously. These symptoms subsided in about half an hour. The other sensitized pig, weight 195 gm., lay prone or squatted on the floor of the box and emitted plaintive sounds. The animals were kept in the stable overnight and appeared wholly normal when removed next morning. The control animals showed no particular disturbance.

Some drops of serum were instilled into the nostrils of the smaller sensitized pig and provoked within a few moments somewhat the same reactions as the animal had manifested in the stable. This experiment was, under these conditions, unfortunately not repeated. More than twenty-four hours after its introduction into the stable and about eight hours after its removal therefrom, the larger sensitized pig was given by the vein nearly 1 c.c. of horse-serum. *There was no marked reaction;* soon after its release from the holder the animal fed as usual. Twenty-five days later this pig received another intravenous injection of serum and died within four minutes. The smaller of the two sensitized pigs received its toxic dose some twenty-eight hours after removal from the stable. Owing to difficulties in manipulation a great excess of serum was injected into the vein, probably more than 1 c.c. The animal did not suffer the usual shock, but died from respiratory failure in about half an hour; its lungs were almost completely collapsed when the chest was opened.

In the light of later observations I am inclined to think that this animal's death was a sign of the passing of the phase of antianaphylaxis induced by inhalation of stable-air combined with the excessive magnitude of the toxic injection. No effort was spared, of course, in seeking a solution of the results obtained in these preliminary experiments. Of fundamental importance appeared to be the question whether the desensitizing agent in the air of the stable affected the body indirectly through action on the olfactory mucous membrane or was absorbed directly through the respiratory tract. To resolve this question, groups of pigs, sensitized or normal, were operated on for section of the olfactory nerves. Under ether, a small hole was trephined just posterior to the cribriform plate, the nerves were cut with a fine bistoury and their stumps wiped out with a small pledget of cotton held in forceps. The animal manifests little or no disturbance after such an operation.

In other animals not only the olfactory nerves were removed but the olfactory lobes as well. But, for reasons that will be detailed later, this more extensive operation was necessarily performed only on normal, non-sensitized animals. The experimental data are condensed in the following protocols:

EXPERIMENT 1.—Four sensitized guinea-pigs, two with intact olfactories, exposed to stable August 18.

Pig 1, male, 480 gm.; olfactory nerves excised July 12; sensitized with serum July 16. No reaction in stable.

Pig 2, male, 645 gm.; sensitized July 16; olfactory nerves excised August 15. Restlessness after twenty minutes in stable.

Pig 3, male, 675 gm.; unoperated, sensitized July 16. Much erotic excitement after fifteen minutes in stable.

Pig 4, male, 750 gm.; unoperated, sensitized July 16. No reaction except slight uneasiness after forty minutes in the stable.

After remaining all night in the stable each animal received, August 19, an intravenous injection of more than 0.5 c.c. of fresh serum. Every pig died in characteristic shock except Pig 3, which responded to the injection with violent struggles, some respiratory râles, deepened respiration and rubbing of the nose when released. After this moderate shock the pig completely recovered.

One cannot fail to connect the excitement of this pig when exposed to the stable air, attempting to copulate with his male companion, etc., with the desensitization certified by the development of antianaphylaxis. This animal was reexposed in the stable thirty-three days later, when he showed but slight excitement, and the following day he succumbed to intravenous injection.

EXPERIMENT 2.—Two pigs received subcutaneous serum July 16. Exposed in stable August 20.

Pig 1, male, 525 gm. Olfactories intact. Within five minutes after entering stable is erotic and continues restless, scratches nose, etc., for an hour.

Pig 2, male, 540 gm. Olfactory nerves had been cut July 12, and a serum inhalation given August 16. In the stable is restless, slightly dyspneic and scratches chin during an hour.

August 21, intravenous injection of serum, more than 0.5 c.c., was given to each. Pig 1 had two slight spasms, sneezed twice and then recovered. Pig 2 showed very little reaction. Result, antianaphylaxis in both animals.

The recovery of the second animal would appear to show that the olfactory apparatus was not concerned in the desensitization, but on August 22, its brain was exposed for the purpose of excising the olfactory lobes; it was then found that the olfactory nerves appeared to have regenerated in the interval of thirty-nine days elapsing between the nerve section and the stable exposure.

EXPERIMENT 3.—Two pigs sensitized July 16. Both had been in the same box with the animals used in a former experiment. They were placed again in the stable for forty minutes, August 22. Twenty hours later they received the toxic injection.

Pig 1, male, 660 gm. Somewhat erotic and restless in stable.

Pig 2, male, 360 gm. Olfactory nerves cut July 12. Restless and rubs nose in stable.

Both died from the toxic injection, which, it will be noted, was given twenty hours after leaving the stable, whereas the usual interval was two or three hours. Necropsy on Pig 2 indicated partial regeneration of the olfactory nerves.

EXPERIMENT 4.—Two pigs sensitized July 16. Placed in stable for five hours, August 25.

Pig 1, 425 gm.; a comparatively feeble animal. Restless, rubs nose and scratches in the stable.

Pig 2, 790 gm.; olfactory nerves were cut, August 24. Less excitement shown in the stable than in the case of Pig 1.

Intravenous injections of more than 0.5 c.c. of serum were given within an hour of removal from the stable. Pig 2 died promptly from shock. Pig 1 showed no reaction except for temporary muscular weakness becoming apparent in an hour. This animal was undoubtedly antianaphylactic but was found dying the next morning after exposure to the cold of the previous night. It was frequently noted that guinea-pigs recovering from the strain of anaphylactic shock are exceedingly non-resistant to external cold. Their temperature regulation is apparently profoundly disturbed.[3]

It is unnecessary to go further into detail along this line of evidence. Suffice it to say that a large proportion of guinea-pigs sensitized subcutaneously to horse-serum were desensitized by exposure to the air of the horse-stable. There was a very obvious relation between the pungency of the stable-odor and the latent period of anaphylactic disturbance. When the smell was most intense the nervous symptoms of anaphylaxis came on almost immediately. In Denver the nights became strikingly cool in August so that the more intense stable-odor only endured for two or three weeks in July and the time required to sensitize guinea-pigs did not allow of prolonged investigation under conditions most favorable to olfactory stimulation.

As the mean temperature of the air fell and the stable odor became less intense the latent period of anaphylactic disturbance increased from a very few minutes to a quarter of an hour, then to longer periods and finally the onset of disturbance could not be distinguished. Nevertheless sensitized pigs kept in the stable for a day or more sometimes

3. Doerr: In Kolle and Wassermann's Handbuch des pathogenen Mikroorganismen, 1913, ii, Part 2, p. 1070.

proved to be antianaphylactic to intravenous injection even when they had manifested no striking symptoms marking desensitization.

All the earlier observations pointed to the conclusion that only pigs possessing intact olfactory mechanisms were subject to desensitization through the stable air. Obviously a single positive result demonstrating antianaphylaxis in the case of a smell-free guinea-pig would show that the noxa producing desensitization could affect the body through channels other than the olfactory. Such a demonstration was afforded by observations on four animals kept in the stable for three days during the first week in October. Each had suffered either recent section of its olfactory nerves or loss of both olfactory nerves and lobes many weeks previously. Submitted to the test of intravenous inoculation, one of the pigs whose olfactory lobes had been excised thirty-nine days before and which had been sensitized by subcutaneous serum thirty-six days before, recovered from the toxic injection after slight symptoms of shock.

This section of my work may be briefly summarized by stating that fourteen sensitized guinea-pigs with intact olfactory sense were exposed to the stable air. Of these, five became completely antianaphylactic to the intravenous toxic injection and two succumbed only after evidence of great resistance to the toxic dose. The fourteen pigs included two that had been previously made antianaphylactic as witnessed by recovery from an intravenous injection; both died from the second intravenous dose. The fourteen pigs also included two in which the olfactory nerves had been cut long before but which seemed to have regenerated.

Ten sensitized pigs were exposed in the stable after recent section of their olfactory nerves or long-standing ablation of the olfactory lobes. None of these had manifested any marked symptoms of anaphylactic disturbance in the stable. Only one of this group survived the toxic intravenous injection.

Daily contact with such phenomena as have been described is bound to give rise to certain more or less defensible impressions which have not been submitted to definite proof. Of this nature are the notions that the desensitizing agent in the stable-air operates on the body in two ways: (1) acutely, as an odor irritating the olfactory nerves with the production of anaphylactic symptoms; (2) more slowly, by absorption through the respiratory tract, without the production of striking anaphylactic symptoms. The important question as to whether antianaphylaxis can be produced by the mere odorous stimulation of the olfactory epithelium has not been settled, although the facts seem to point in that direction. The phase of antianaphylaxis in these experiments, as found in other fields, comes on very soon after exposure to the exciting cause. Also, several experiments pointed to the conclusion

that the antianaphylactic period is very short — that it is rapidly vanishing within twenty-four hours after removal from the desensitizing environment.

The theory having been proved that fully developed sensitization could be temporarily undone by exposure of animals to stable air, the conclusion seemed obvious that sensitization should be altogether prevented in pigs kept in the stable during the latent period of sensitization. The result of an appropriate experiment proved, however, directly the contrary, as follows:

EXPERIMENT 4.—Five guinea-pigs, weighing about 350 gm. each, were placed in the stable October 24, about twenty hours after receiving subcutaneous injections of serum, with the exception of one animal which had received an injection ten days before. This last pig was taken from the stable October 29, that is, fifteen days after its sensitizing injection and at the end of five days' residence in the stable. An intravenous injection given at this time was fatal within four minutes. The remaining four pigs were taken from the stable November 17, after exposure of fourteen days, and each succumbed to an intravenous injection within about three minutes. Necropsy was performed on these, as in all other fatal cases, and the lungs were found in characteristic anaphylactic distention. Guinea-pigs of this group manifested a somewhat striking difference in behavior from all other animals exposed in the stable. They never recovered from the timidity associated with the change of environment, nor did they acquire a normal appetite for food. The results of the experiment indicate that, at least in cool weather, the processes leading to sensitization go on in the environment which may effect desensitization of a fully sensitized animal.

SENSITIZATION TO HORSE-SERUM IS NOT CAUSED BY EXPOSURE TO STABLE AIR

As to the nature of the agent producing the desensitizing effects, I have no definite evidence. When the box containing the guinea-pigs was held directly under the nose of a horse, or when the manure-impregnated straw of the stable was placed within it no particular reaction was observed. An effort was made, of course, to sensitize the guinea-pigs to horse-serum by the same influence which caused their desensitization.

The two normal pigs which had been used as controls and exposed to stable air, July 7, each received an intravenous injection of 0.5 c.c. of horse-serum sixty-three days later. There was nothing like shock to be discerned, though one of the animals rubbed its nose vigorously ten minutes after its release, the other seemed sleepy and both preened the body as if stimulated by itching.

Four normal male pigs, weighing about 390 gm. each, were placed in the stable, September 9. One of them was removed after twenty-four hours and within an hour thereafter received an intravenous injection. There was no reaction except that the animal remained rather unusually quiet. For fourteen days the remaining pigs were kept in the stable,

two of them being taken out for some hours each day. One of these and the third pig — which had been continuously in the stable — were given intravenous injections within about an hour after removal, with completely negative results. The evidence shows that while the possibility of minor degrees of sensitization to toxic injections of serum cannot be denied, nothing like the sensibility leading to fatal shock is induced.[4] The slighter degrees of anaphylactic reaction are somewhat uncertain in significance since the normal pig is prone to manifest the same actions, such as nose-rubbing and preening the body.

ANAPHYLACTIC SHOCK AND DESENSITIZATION CAUSED BY OPERATIONS ON THE BRAIN OF THE SENSITIZED GUINEA-PIG

According to the well-known views of Besredka,[5] sensitization in the guinea-pig is primarily or wholly a property of the brain. This author holds the view that during the process of sensitization the body tissues form a substance *une sensibilisine,* which fixes itself to the brain-cells. Intracerebral injection of a large dose of serum (0.25 c.c.) produces rapid desensitization with great shock or death. With small intracerebral injections (0.0025 c.c. to 0.025 c.c.), desensitization of the cerebrum comes gradually and the animal becomes antianaphylactic. The animal is resensitized by a new injection made within this period. He holds that the condition of antianaphylaxis is a return to the normal state. Guinea-pigs may be desensitized by intracerebral injections but cannot be sensitized thereby, that is, the brain-cells cannot prepare the sensitizing substance. In an animal thoroughly narcotized by ether, the intracerebral injection of serum produces no shock but still induces antianaphylaxis.

That brain sensitization cannot altogether account for the phenomena of anaphylactic shock was sufficiently demonstrated by Auer and Lewis,[6] who found that sensitized guinea-pigs were killed by intravenous injection and showed characteristic distention of the lungs after section of the vagi nerves and after destruction of the base of the brain, medulla and spinal cord. That the irritability of the brain is profoundly modified by the state of sensitization was early made clear in our experiments.

In attempting to remove the olfactory sense from sensitized pigs in preparation for the stable experiments, it was found that while intracranial section of the olfactory nerves produced no particular reaction,

4. Not until this work had been long completed did I learn that Rosenau, in an announcement I have not been able to trace, describes successful efforts to sensitize guinea-pigs in a horse stable.

5. Besredka, A., and Steinhardt, E.: Ann. de l'Inst. Pasteur, 1907, xxi, 117; Besredka, A.: Ann. de l'Inst. Pasteur, 1907, xxi, 777 and 950; ibid., 1910, xxiv, 273, 879, etc.

6. Auer and Lewis: Jour. Exper. Med., 1910, xii, 151.

ablation of the olfactory lobes caused profound disturbance, as illustrated in the following experiment:

EXPERIMENT 5.—Four guinea-pigs, about 730 gm. each, were given subcutaneous injections of 0.33 c.c. of serum, July 16. August 15, under light ether anesthesia, the olfactory nerves were cut and wiped out in three animals; there was no special reaction. In the fourth pig, which received the least ether, both olfactory lobes and nerves were removed. After its release the animal continued to lie on its side and in about fifteen minutes severe clonic convulsions of the limbs appeared, lasting half an hour or more. The animal gradually recovered, but more than two hours after the operation manifested rhythmic "bucking" movements of the body without the feet leaving the floor. Next day this animal was well and was given 0.75 c.c. of serum intravenously; there was no reaction except for slight torpor. One of the first three pigs was given a like injection immediately afterward and died within three minutes.

It should be said that removal of the olfactory lobes in a normal guinea-pig produces no definite response. The shock and subsequent antianaphylaxis described are no special effect of removal of the olfactory lobes, but follow mutilations of the cerebrum, thus:

EXPERIMENT 6.—A vigorous male pig, 700 gm., was sensitized July 16. August 23, under ether, an amount of cerebral tissue in bulk about that of the olfactory lobes was removed from the parietal regions. The animal when released continued for ten minutes to lie on its side with clonic twitching of the hind legs. The next day this pig received an intravenous injection of 0.75 c.c. of serum and, with the exception of slight asthmatic wheezing, showed no special reaction.

EXPERIMENT 7.—Male pig, 780 gm., sensitized August 14. August 21, a bulk of cerebrum in size five or six times that of the olfactory lobes, was removed. For nearly an hour the pig lay on its side with clonic twitchings, increasing to convulsions, of the legs. It sprang up and bucked after sitting up and then for many hours carried on continuous coordinated forced movements, seeking always to escape restraint and wander forward. The next morning the animal was found dead. The fatal result was probably brought about partly by failure to react to the lowered temperature of the night air.

Numerous experiments of like character have definitely proved that anaphylactic shock is caused by mutilation of the brain in the sensitized guinea-pig. The degree of shock seems rather strictly related to the amount of cerebral tissue removed, but varies somewhat in different animals. The intensity of shock suffered after a definite nerve injury seemed to diminish as the weather became cooler. If this impression is confirmed it may be elucidated by the facts portrayed in the next section of this paper. In the accounts[7] of anaphylactic shock and the resulting phase of antianaphylaxis usually given, the phenomena have been induced by reinjection of the antigen which caused sensitization, but here are examples of profound somatic changes being induced by mere ablation of brain-tissue in amounts whose loss would cause no particular disturbance in a normal animal. In seeking an explanation

7. Doerr: In Kolle and Wassermann's Handbuch der pathogenen Mikroorganismen, 1913, ii, Part 2, p. 1085.

for the facts it seemed probable that the effective stimulus consisted in the mechanical agitation of the brain. To test this view an electric vibrator, designed for therapeutic massage, was used, its flat ebonite disk, rapidly oscillating, being firmly applied for three minutes to the skulls of a series of sensitized guinea-pigs which were slightly etherized. The results showed a curious discrepancy.

EXPERIMENT 8.—A guinea-pig previously described, which had received a subcutaneous injection of serum July 16, had lost part of its cerebrum August 23, and had recovered from an intravenous injection August 24, was submitted to skull vibrations September 22. The only reaction to the vibration was rubbing of the nose and a slight "steppage" movement. September 23 more than 0.5 c.c. of serum was given intravenously, resulting in dyspnea, peristalsis and cough, but recovery within an hour. (This animal received another vibration November 9, followed by intravenous injection November 11, from which it recovered. Another intravenous injection was given fourteen days later with recovery, and a fifth injection into a vein was made December 9, without producing symptoms. This is a case of either prolonged antianaphylaxis or permanent immunity.)

EXPERIMENT 9.—Guinea-pig, male, 820 gm., sensitized July 16. From August 16 to August 31 it received five inhalations of vapor of horse-serum without effect. September 2 the electric vibrator was applied to its skull for about five minutes. There was no reaction except "steppage" movements, nose rubbing and slight incoordination. The next day the animal received intravenously 0.75 c.c. of serum and fully recovered with very slight symptoms. (Twenty days later the olfactory lobes were removed. The shock was very intense. The animal never fully recovered, though it would eat greedily; it died four days after the operation.)

The results of subsequent experiments indicate that desensitization caused in the foregoing animals by vibration of the skull was dependent in some way on the treatment they had previously received auxilliary to the primary sensitization. Five other guinea-pigs, two of which had been sensitized intravenously, were submitted to vibration and all died in shock when injected the following day by the vein. Two of the animals before the toxic injections received no less than six vibration-treatments at intervals of two days. The application of the vibrator to the skull is exceedingly drastic treatment and frequently resulted in complete inhibition of the respiration and great slowing and enfeeblement of the heart-beat.

The phase of antianaphylaxis produced by the shock of brain operations is very short. It passes off in less than forty-eight hours, as was evidenced by three special experiments which it is not necessary to detail. That no shock is occasioned by moderate ablation of nervous tissue in the normal pig is shown by the following:

EXPERIMENT 10.—Normal male, 360 gm. Olfactory lobes and a portion of the anterior cerebrum slightly greater in bulk were removed November 3. There was no reaction whatever. (In two days this pig sickened, probably from infection, and died in a week. Other normal pigs suffered loss of much greater quantity of brain without permanent disturbance).

In a widely quoted paper, Schürer and Strasmann[8] describe experiments in which they attempt to contravert Besredka's idea that the sole seat of the anaphylactic reaction is in the brain. They excised the whole brain, including the basal ganglia, in sensitized guinea-pigs and then gave them serum by intravenous injection. The animals died within a period varying from a few minutes to several hours, and the results were supposed to contravert the theory that the brain is involved in anaphylactic phenomena. In the light of my observations it is plain that such methods are necessarily incompetent to clear up the subject.

DESENSITIZATION OF THE BRAIN THROUGH SECTION OF THE OLFACTORY NERVES

On one occasion, the olfactory lobes were removed from a fully sensitized guinea-pig but the animal manifested no sign of shock whatever. In seeking the cause for this departure from the rule that had seemed established, it was found that this pig had, eight days before, been subjected to section of its olfactory nerves. It at once became necessary to follow out the suggestion aroused by this development.

EXPERIMENT 11.—Six young pigs, weighing about 270 gm. each, were sensitized by subcutaneous injection August 20. From four of these, Pigs 1, 2, 3 and 4, the olfactory nerves were cut and wiped out September 16, under very slight etherization. There was no reaction except to a very slight degree in one (Pig 2) in which bleeding occurred through the nose due to injury to the cribriform plate. September 24, twenty-six days after subcutaneous injection, the olfactory lobes were removed from Pigs 1, 2 and 5 (the latter having intact olfactory nerves). Pigs 1 and 2 showed no shock; Pig 5 lay on its side for nearly ten minutes with somewhat convulsive clonic trembling of the legs. This shock did not produce antianaphylaxis for the animal succumbed to intravenous injection the next day. September 25 the olfactory lobes were excised from Pig 3. There was no shock whatever, the animal sitting up at once when released, though it rubbed its nose and preened the body. On the same date there were taken from Pig 4 (olfactory nerves destroyed September 16), both the olfactory lobes and a portion of the anterior cerebrum, about equal in bulk to the combined olfactory lobes. The animal lay quietly on its side for two minutes after release from the holder, breathing hard but without convulsive movements, and then sat up with normal appearance except for slight temporary spasmodic nodding of the head. No antianaphylaxis was produced, for the animal succumbed to an intravenous injection the next day, September 25. Pig 6, sensitized but with intact olfactory nerves, was made to lose its olfactory lobes and a portion of the anterior cerebrum equal to a single olfactory lobe. When released this animal lay on its side for five minutes with clonic convulsions. It then sat up and appeared normal for awhile, but soon became restless and the subject of forced movements, which continued until the animal died in convulsions, attended with enormous erections of the penis, after six hours. At necropsy the lungs were found collapsed, containing a few hemorrhagic areas but no consolidation.

8. Schürer, J., and Strasmann, R.: Ztschr. f. Immunitätsforsch., 1912, xii, 143.

The experiments show very definitely that in sensitized guinea-pigs the nervous shock following mutilation of the brain is greatly reduced eight or ten days after section of the olfactory nerves. It was sought to determine how soon such desensitization of the brain followed on nerve section, and how long it continued.

EXPERIMENT 12.—Pig 1, male, 440 gm., sensitized August 29. Olfactory nerves destroyed September 24. Next day the olfactory lobes were removed. The animal manifested moderate but decided action, such as relatively slow recovery from the operation followed by nose-rubbing and clonic tremblings of the body. The shock was not sufficient to produce antianaphylaxis for the pig succumbed in three minutes next day to toxic injection.

Pig 2, female, 910 gm., far advanced in pregnancy, sensitized August 29. Olfactory nerves cut November 5. November 6 the olfactory lobes and a like bulk of cerebrum were removed. There was scarcely any shock following the operation, the only sign of anaphylaxis being a slight inspiratory humping of the body setting in ten minutes after release.

These results indicate that while the desensitizing effect of olfactory nerve section on the brain may develop within a day, the full influence of the deprivation of olfactory stimuli is not established with certainty till later.

EXPERIMENT 13.—Male pig, 500 gm., sensitized August 29. Olfactory nerves cut November 5. November 18 excised olfactory lobes and a similar bulk of cerebrum. The animal lay quiet for nearly ten minutes after release and then sat up with no evidence of reaction except for slight rhythmic lifting of the legs. Next day the pig succumbed in three minutes to the toxic injection.

It is clear from these experiments that the diminished sensibility of the brain following section of the olfactory nerves in sensitized guinea-pigs may be well marked within twenty-four hours and remains in full force for at least thirteen days. After longer periods there may always be suspicion of regeneration of the olfactory nerves. In the following experiments part of the cerebrum was removed long after ablation of the olfactory lobes:

EXPERIMENT 14.—Female pig, 690 gm. Olfactory lobes removed August 27 and the space left filled with melted paraffin (in none of several cases in which this was done could the paraffin be detected with certainty after several weeks). Serum was injected subcutaneously August 29. November 3, sixty-eight days after removal of the lobes, under slight ether anesthesia, there was scooped from the anterior cerebrum an amount of tissue estimated as about twice the bulk of the normal olfactory lobes. There resulted moderate but distinct shock, with some forced movements, which passed off after about thirty minutes. Two days later the animal withstood an intravenous injection of nearly 0.5 c.c. of serum with scarcely any reaction.

This experiment indicates that after removal of the olfactory lobes (of course including the attached nerves) the sensitiveness of the brain to laceration has been more or less completely restored in about two months. Similar conclusions were derived from other experiments the

conditions of which were more complicated, which it is unnecessary to describe.

It might be claimed that the influence on the irritability of the brain apparently exerted by the olfactory sense is not a specific property of this sense but is wielded by any strong sensory impulse or is also a part of the function of other special senses. I did not fully grasp, until too late to sensitize new animals, the importance of testing the relation of the visual sense to cerebral irritability. A single experiment, however, went to show that the removal of one eye does not desensitize the brain as in the case after section of the olfactory nerves.

EXPERIMENT 15.—Male, 615 gm. Sensitized by intravenous injection of 0.5 c.c. of serum September 6. Under ether anesthesia left eyeball was removed November 6. November 10, under ether, the olfactory lobes and an equal bulk of cerebrum were removed. There was moderate but pronounced shock lasting for more than half an hour. The shock was not sufficient to protect the pig with anti-anaphylaxis when an intravenous injection was given next day.

From the results that have been described in the last two sections of this work it seems impossible to avoid the conclusion that sensitization of the brain and sensitization of the body represent different biologic conditions. The former is a labile, the latter a stabile, state. The threshold of irritability of the sensitized brain is plainly leveled in response to olfactory sensory stimuli. The brain may be completely desensitized without affecting in the least the response of the body to the second, toxic dose of the antigen. An attempt to elucidate the teleological significance of these coordinations will be made in the discussion at the end of this paper. As is well known, Besredka asserts that the intracerebral injection of 0.25 c.c. of serum will, in an unanesthetized sensitized guinea-pig, cause speedy anaphylactic death. If the conclusions drawn above as to desensitization of the brain following section of the olfactory nerves be correct, it should be possible for an animal so treated to resist Besredka's fatal injection. That this is true is proved by the following:

EXPERIMENT 16.—Pig 1, female, 360 gm., sensitized August 29. Olfactory nerves cut and wiped out September 16. September 26, under light ether anesthesia, a trephine hole was made over the cerebrum and there was injected through a hypodermic needle into both hemispheres a total of 0.25 c.c. of horse-serum. The animal was practically out from under the influence of ether during the injection. There was but very slight reaction marked by restlessness and some jerking of the head. This animal weighed 450 gm. November 5, when it received an intravenous injection and died in four minutes.

Pig 2, female, 840 gm., sensitized August 29. Olfactory nerves cut November 5. November 8 the skull was trephined over posterior cerebrum and the animal was released until entirely recovered from ether; then, as before, 0.25 c.c. of serum was injected into the brain. There was rather severe shock marked by clonic twitchings of legs, rapid breathing, circular movements, weakness of hind legs; gradual recovery after an hour or two. This shock was not sufficient

to produce antianaphylaxis, for the animal succumbed next day in the usual way to intravenous injection.

Besredka found that intracerebral injections made with very small amounts of serum, or made under deep ether anesthesia, are not lethal, and the following day the animal is found to be antianaphylactic. Rosenau and Anderson[9] repeated these experiments on etherized guinea-pigs and found that seven out of eight animals died from the second injection given next day. These discrepancies may be accounted for by supposing that the duration of the antianaphylactic state may be less as well as more than twenty-four hours.

According to the preceding argument, it should be suspected that ether narcosis affects only the susceptibility of the brain to shock, leaving that of the body unaffected. It was found, in fact, that a sensitized guinea-pig etherized to relaxation, died as quickly from intravenous injection of serum as one treated without an anesthetic. In the earlier experiments of this work the intravenous injections were all given without an anesthetic for fear of masking the anaphylactic symptoms; but in all the confirmatory tests the animals were subjected to a slight etherization, with little or no distortion of reactive symptoms.

Reference was made in the preceding section of this paper to an impression which developed as the result of prolonged experimentation, that the anaphylactic shock caused by the ablation of a definite mass of brain tissue became less intense as the weather turned cooler. Such a result might be expected if the olfactory sense, whose excitation probably varies with the external temperature, be indeed, a physiological regulator of brain irritability.

In his excellent contribution to the great work edited by Kolle and Wassermann, Doerr summarizes his discussion on the site of anaphylaxis thus: "That the real points of attack of the anaphylactic noxa lie in the periphery has been established beyond all doubt."[10]

THE RELATION OF THE OLFACTORY NERVES TO SHOCK IN THE NORMAL BRAIN

The marked dependence of anaphylactic disturbance following operations on the brain of serum-sensitized guinea-pigs on integrity of the olfactory nerves raises the question whether in the normal animal there is not a specific physiological relation between the olfactory sense and irritability of the brain. The literature of cerebral physiology makes it obvious that so profound a problem can only be solved by a great

9. Rosenau and Anderson: Jour. Med. Research, 1908, xx, 37.

10. "Das die wesentlichen Angriffstellen der anaphylaktischer Noxa in der Peripherie liegen, ist heute wohl über allen Zweifeln sichergestellt." Doerr in Kolle and Wassermann's Handbuch der pathogenen Mikroorganismen, Ed. 2, ii, Part 2, p. 1078.

number of guarded observations. Nevertheless, a few experiments were ventured in this field and the results were sufficiently positive to deserve record.

Five guinea-pigs were taken which were in very fair condition although they had been inoculated with what was evidently an attenuated strain of tubercle bacilli nearly three months before. In three of the animals the olfactory nerves were cut and two or three days later the olfactory lobes were excised together with a quantity of cerebral tissue estimated at three times the bulk of the lobes. In making this estimate the olfactory lobes were placed on a sheet of paper and three heaps of equal size, scooped from the anterior cerebrum, were laid near by. The operations were performed, as usual, under very light anesthesia. As will be seen, the symptoms of shock were much more pronounced in the two animals whose olfactory nerves remained intact up to the time of the operation.

EXPERIMENT 17.—Pig, 1, weight 600 gm. Excised olfactory nerves November 13. November 15 excised olfactory lobes and about three times their bulk of cerebrum. Pig when released lay for ten minutes apparently conscious, but with clonic tremblings of the legs and slight dyspnea. Recovered quickly; result, very slight reaction.

Pig 2, 485 gm. November 13 olfactory nerves cut. November 16 the olfactory lobes and part of the cerebrum removed as before. The animal lay quietly for six minutes and then sat up without reaction except for some dyspnea, nose-rubbing and rhythmic nodding of the head. Result, very slight reaction.

Pig 3, 510 gm. Olfactory nerves cut November 16. November 18 olfactory lobes and three times their bulk of cerebrum removed. Animal tried to sit up in six minutes after release. There were no convulsive movements, but the animal ran about the room and sought to hide in a dark corner. Normal but dull within an hour. Result, slight reaction as shown by apparently forced movements.

Pig 4, 510 gm. Olfactory nerves intact until time of the main operation November 15, when the olfactory lobes (with nerves) and three times their bulk of cerebral tissue were removed. Pig lay for more than ten minutes after release with clonic tremblings. It then turned on its back with convulsive doubling of the body. It was still unable to sit up in twenty minutes after which there was a convulsive twisting of the body with legs kicking. It soon recovered. Result, marked but moderate reaction.

Pig 5, about 580 gm. Olfactory nerves intact until main operation November 16, when the olfactory lobes and cerebral tissue were excised as before. Pig lay for ten minuntes after release with clonic tremblings of the legs; then there was a tetanoid twisting of the body. Sat up in twenty minutes and manifested apparently forced twisting movements. Within forty minutes the only disturbance was shown by rhythmic lifting of the body. Result, marked but moderate reaction.

In answer to the objection to the use of ether in these experiments it may be said that narcotization was of such light degree that animals in which the olfactory nerves alone were cut would sit up at once without reaction on release from the holder. When nerve-cell tissue was excised the animals lay on the side, with or without convulsive twitchings, for

a period apparently proportional to the amount of substance removed; that is, the symptoms were a result of the operation, not of the anesthesia.

INFLUENCE OF COCAINIZATION OF THE NOSE ON THE SHOCK FROM INTRACRANIAL OPERATION

The suggestive surgical conceptions of Crile[11] are recalled by these results and an effort was made to modify the shock of brain operations through an attempt to cocainize the olfactory mucous membrane. It is well known that a solution of cocain sprayed into the nose may completely abolish the sense of smell temporarily. Only a few unconvincing experiments were tried in this direction. A 2 per cent. solution of cocain was dropped into the nostrils of two sensitized guinea-pigs four times in the course of fifteen hours. Then under light ether anesthesia the olfactory lobes and a somewhat greater amount of cerebral tissue were removed from each, with resultant moderate but distinct reaction in each. The shock was much less and cleared up sooner than that which occurred in most sensitized pigs subjected to the same operation without cocain. Each of these animals succumbed next day to intravenous injection. Of four young pigs weighing about 240 gm. each, from a sensitized mother, two were treated with several nasal instillations of cocain. One of these pigs quickly died with symptoms to be detailed later. The other three were subjected to the loss of the olfactory lobes and about three times their bulk of cerebral tissue. There was decidedly less prolonged convulsive reaction in the cocainized pig than in the untreated pigs, though the former showed a high degree of nervous excitement. From the results of observations already detailed it is obvious that cocain anesthesia should be prolonged for a day or two in order to obtain its desensitizing action, if any, on the brain.

CERTAIN ANALOGIES BETWEEN THE EFFECTS OF COCAIN POISONING AND ANAPHYLACTIC SHOCK

Guinea-pigs were killed by nasal instillation of cocain and by subcutaneous and intravenous injection. Five unsensitized pigs were given intravenous injections of cocain in about 4 per cent. solution. Whether the poison was injected slowly or rapidly the result was the same, a speedy diminution in the amplitude of respiratory movement, and death without convulsion. The heart continued beating for many minutes after respiratory arrest just as it does in anaphylactic death. The lungs were found collapsed at necropsy.

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11. Crile, G. W.: Boston Med. and Surg. Jour., 1910, clxiii, 893.

Three young pigs, offspring of a sensitized mother, weighing 180 to 240 gm. each, were killed by the repeated instillation in the nose of a cocain solution of from 2 to 4 per cent. The symptoms of poisoning came on very suddenly. The animal would fall on its side; the head was tonically retracted; frequent and well-marked opisthotonus and clonic convulsions of legs and body occurred. The same series of events occurred when subcutaneous injections of cocain were given. This was done in the case of seven animals, four of which were serum-sensitized, but the element of respiratory disturbance was more marked than when the poison was given by the nostrils. The retraction of the head was a characteristic symptom, the chest would be distended and the respiratory movements very rapid and so shallow as to be hardly visible. An animal might recover after remaining more than half an hour apparently *in extremis*. The convulsive phenomena witnessed in these animals were exceedingly suggestive of those seen in severe anaphylactic shock. But the particular object of these experiments was to follow out an observation that had been made on the pig from a sensitized mother, which had first succumbed to cocain by the nose. The lungs of this animal were found, post mortem, to be markedly distended, almost as much so as after anaphylactic shock. In two other cases this extraordinary post-mortem distention of the lungs was found, although usually the lungs were well retracted from the heart on the ventral side but maintaining apparently a condition of semi-expansion dorsally. In two normal guinea-pigs killed by cocain subcutaneously the post-mortem lung-distention was less striking than in the sensitized or offspring of sensitized pigs. That the post-mortem distention of the lungs did not depend on the terminal convulsions seemed evidenced by the state of two sensitized pigs which were injected with strychnin; in these the lungs were found completely collapsed at necropsy. The expanded lung after death from cocain offers marked differences from the expanded lung after anaphylactic death. The former is pale, the latter congested. Both remain distended when excised, but the former crepitates little and collapses but slightly when squeezed in the fingers; the latter does both. In that minority of cases in which the lung remains distended after death by cocain, its behavior suggests a rubber-like structure. The phenomena described in this section recall the similar, though still more striking, effects described by Dale and Laidlaw[12] as following the injection into guinea-pigs of betaimidazolylethylamin. They call attention to the anaphylactic-like changes produced by this substance.

12. Dale and Laidlaw: Jour. Physiol., 1911, xliii, 182.

REACTIONS OF GUINEA-PIGS TO HORSE-SERUM ADMINISTERED THROUGH THE NOSE AND BY INHALATION IN SPRAYS: LOCAL AND GENERAL ANAPHYLAXIS

Comparatively few investigations seem to have been made with a view to determining the effect of the application of antigens to the unaltered peripheral epithelium. The reaction to the tuberculin test of von Pirquet involves wounding of the outer skin. Administration of antigens by feeding, especially by means of the stomach-tube, greatly complicates the experimental conditions. In an important paper, Dserzgowsky[13] describes experiments in which plugs of cotton wool moistened with the toxin of diphtheria were applied to the mucous membrane of the nose and the toxin itself to the general respiratory tract through the inhalation of sprays. Experiments were performed on horses and on the author himself. It was asserted that in these ways active immunity, both local and general, could be harmlessly induced. Blumenau,[14] following this suggestion, inserted for thirty minutes tampons moistened with diphtheria toxin, diluted to one-third strength, into the nostrils of children. After twenty such treatments diphtheria antitoxin was found in the blood. Practically the same result was obtained by Scheweleff[15] in experiments on dogs. A. C. Klebs,[16] in his history of small-pox inoculation, mentions an ancient custom among the Chinese of protecting against small-pox by putting its virus into the nose.

Busson[17] describes experiments in which guinea-pigs sensitized to horse-serum were inclosed in a tight box and made to inhale a finely divided spray of horse-serum, ten times diluted, for a period of two hours or more. As far as the description can be understood there were very slight but positive signs of anaphylactic reaction. The rise of temperature within the box and the deterioration of the quality of the air must have introduced important complications into the experiment. My results confirm, in general, those of this author.

Schlecht[18] records a series of researches which show that injection of a foreign serum into the body causes in the blood a great temporary increase of eosinophils, and in guinea-pigs of mast-cells as well, due to increased activity of the bone-marrow. The administration of spray

13. Dserzgowsky, S. K.: Ztschr f. Immunitätsforsch (abst.), 1910, iii, 602.
14. Blumenau, N. R.: Ibid., 1910, iv, 353.
15. Scheweleff, P. P.: Ibid., 1910, x, 1085 (Ref. Bd.)
16. Klebs, A. C.: Bull. Johns Hopkins Hosp., 1913, xxiv, 69.
17. Busson, B.: Wien. klin. Wchnschr., 1911, xxiv, 1492.
18. Schlecht, H.: Kong. f. inn. Med., 1912, xxiv, 416; Deutsch. Arch. f. klin. Med., 1910, xc, 308; ibid., 1912, cviii, 405; Arch. f. exper. Path. u. Pharmakol., 1912, lxvii, 137.

inhalations of horse-serum to guinea-pigs, either sensitized or normal, was followed by a high grade of eosinophilia localized in the lungs.

In the discussion of Schlect's paper, read before the Congress for Internal Medicine, Schittenhelm said that he had not been able to repeat the results of Friedberger, who had produced aseptic pneumonia in sensitized guinea-pigs by inhalations of serum.

Ishioka[19] made injections of serum through a fine cannula directly into the trachea of sensitized guinea-pigs. Out of forty animals two died at once in anaphylactic shock; the others showed symptoms but recovered. In a few cases there was produced interstitial pneumonia with much emphysema. In most cases there resulted true lobar pneumonia indicated by patches of infiltration which could even involve a whole lobe.

My method of bringing the serum in contact with the respiratory epithelium differed radically from that described elsewhere. Some two dozen guinea-pigs were treated in two groups; in one the treatment consisted in instilling fresh horse-serum into the nose by dropping it from a hypodermic needle; in the other the serum was breathed as a fine spray. In the first case the animal was gently held by the hand on its back; from an all-glass hypodermic syringe with easily moving piston and a coarse, blunt needle, droplets of serum to the amount of from 0.25 to 0.33 c.c. were allowed to fall into one or the other of the nostrils. As a rule, only one side of the nose was utilized at each sitting. Various animals were treated daily or at intervals of one, two or three days.

The serum spray was produced in a commercial "vaporizer" actuated from a compressed-air tank. The spray thus formed was exceedingly fine and resembled the vapor from a gently boiling teakettle. A funnel made of stiff paper was applied over the nose and mouth of the guinea-pig as it sat on a table. The nozzle of the spray apparatus was closely applied within the smaller but open end of the funnel. The spray was applied at intervals similar to those of the instillations and the sittings lasted, as a rule, about three minutes. The serum was often used in full strength; in dilutions of one-half or one-quarter strength the results appeared the same. Theoretical objections against this method of procedure seem invalid in the light of the reactions obtained.

Each group of guinea-pigs was composed of four subdivisions: (1) normal animals; (2) animals sensitized by the subcutaneous injection of serum; (3) unsensitized animals which had weeks before been relieved of their olfactory lobes; (4) animals which, after the removal of their olfactory lobes, had been subcutaneously sensitized.

19. Ishioka, S.: Deutsch. Arch. f. klin. Med., 1912, cvii, 500.

SPECIAL REACTIONS TO THE NASAL INSTILLATION OF SERUM

When horse-serum is slowly dropped as described into a nostril of a guinea-pig for the first time, there is usually no disturbance whatever. The fluid is quickly sucked into the nose, and not until a considerable quantity has been introduced is there any expulsion, and this is evidently mechanical. Late in the sitting, taste and probably swallowing motions are manifested. The nasal canal in a medium-sized guinea-pig has a length of nearly 5 cm. and therefore it is not likely that the first droplets reach the pharynx. Even with the second treatment, after two days, signs of active resistance to the instillation of serum may appear, brisk expiratory sniffing movements more or less resist the entrance of serum or expel it from the nostril.

These minor phenomena are physiologically significant but are difficult to interpret because they may occur with the instillation of normal saline or solutions of cocain at the very first administration. It was noted, however, that active resistance to the instillation of serum appeared uniformly earlier in animals with intact olfactories than in those deprived of their olfactory lobes. In animals treated every alternate day, my notes record signs of great nasal irritation in normal pigs at the second instillation, while in specimens without olfactory lobes it was not until the fourth treatment, after six days, that active reactions were produced.

Previous subcutaneous sensitization had no perceptible influence in accelerating the reactions. One definite and undoubtedly reflex response to the instillation was regularly obtained from certain animals. This was an excess of salivary secretion. While the mouths of practically all pigs which showed signs of irritation against the serum became unusually moist, in a certain number saliva would well up between and drool from the lips. This reaction held constant in the same pigs through a prolonged series of observations. The reaction was usually positive in normal pigs. It was obvious, however, that saliva accumulating in the mouth was frequently removed by swallowing and thus failed to be noted. The reaction can hardly be regarded as other than a sign of specific sensitization. It occurred both in animals previously subcutaneously sensitized and in unsensitized animals, whether with or without olfactory lobes. It did not occur through the instillation of normal salt solution even when it was constantly produced by serum. A single nasal instillation with serum evidently induces sensitization. Thus, October 11, two normal guinea-pigs each received 0.25 c.c. of serum in both nostrils. The only effect witnessed was the excitement of taste movements.

Sixteen days later, October 27, the treatment was repeated. There was now considerable nasal irritation as shown by sniffing and active

expulsion of serum from the nose and rubbing of the nose with the paws, and in each case saliva welled from the mouth. Two days later, October 29, one of these animals received an intravenous injection of serum; there was *no reaction.* Five other nasal instillations were given to each pig from November 12 to November 20. The resulting symptoms were the same as those elicited October 27, except that now the animal which had not received the intravenous injection ceased to respond to the instillation by the secretion of saliva. Finally, November 23, both animals received intravenous injections and both died in typical shock. This account anticipates my discussion, but clearly indicates that *local sensitization of the nasal mucous membrane may occur independently of and precede general sensitization.*

The symptoms thus far described as resulting from nasal instillation of serum are minor reactions which it is possible to confuse with those following non-specific stimuli. But there were extraordinary results obtained in the case of a single animal, which, though obviously somewhat dependent on idiosyncrasy, are detailed because they differ only quantitatively from reactions characteristic of this group of experiments. A guinea-pig weighing 435 gm. was treated by the nostrils every second day. During the tenth treatment, eighteen days after the first, the animal suddenly choked; loud rattling râles with occasional cough accompanied the deep and labored respiration. The phenomena strongly suggested those of the severest form of bronchial asthma in man. It was several hours before the pig recovered sufficiently to eat. It had been noticed that spasmodic partial closure of the nostril had resisted the intake of serum. Subsequent administrations of serum each second day provoked similar though milder symptoms, an excessive salivary secretion becoming a relatively more prominent reaction. The symptoms could not have been due to the flow of serum directly into the trachea, for they appeared while still too small a quantity of serum had been applied. Later results with other animals leave no doubt as to the anaphylactic character of the disturbance.

THE EFFECT OF THE INHALATION OF SERUM SPRAYS

The statement was made in the beginning that the initial exhibition of vaporized serum, according to the method described, provoked no symptoms whatever. Guinea-pigs previously sensitized by subcutaneous injection reacted apparently as negatively to the inhalation as did normal animals.

A remarkable difference, however, presented itself in the behavior of those guinea-pigs which received the serum inhalation after a course of serum instillations by the nostril. In these cases there was an immediate response to the first inhalation in the form of a violent

dyspnea, with occasional cough, asthmatic in its urgency but characterized by rapid respiratory movement. The attack would develop suddenly within about a minute of beginning inhalation and would not subside for from thirty minutes to several hours. Unfortunately no attempt was made to determine whether a phase of antianaphylaxis succeeded recovery from the attack of dyspnea. Repetitions of inhalation at intervals of two days or more invariably provoked the same response.

EXPERIMENT 18.—Two young pigs were treated by instillation of serum in the nose every third and every fourth day from May 28 to July 12. Serum inhalations were given to one July 17, and to the other August 7. Both animals exhibited the reaction described, namely, violent, rapid dyspnea, interrupted with occasional deep breaths and cough.

These, together with subsequent observations, make it clear that local sensitization of the nasal mucous membrane to horse-serum may be induced without the excitement of any very marked reaction. An animal thus peripherally sensitized responds immediately to serum inhalation with characteristic dyspnea, while one sensitized subcutaneously does not. My friend Dr. Hillkowitz kindly tested the blood drawn from the heart of one of the animals described, for precipitins to horse-serum. The dilutions used were 1 : 1,000 and 1 : 10,000. The results were negative, as might have been expected from the similar observations of Rosenau and Anderson.[20] An excellent opportunity seemed presented in these animals to determine whether the olfactory mechanism played any rôle in the serum reactions.

EXPERIMENT 19.—A female pig, 645 gm., which had regularly responded to the inhalation, had its olfactory nerves divided August 25. Four days later, and during subsequent exposures, the serum inhalations produced symptoms identical with those produced when the olfactory nerves were intact, though possibly the latent period of reaction was longer. September 2 the olfactory lobes were excised in this pig without special reaction (because of previous section of the nerves). Two days later a serum inhalation provoked the characteristic dyspnea which began after the vaporization of thirty or forty drops of serum. September 10 this animal succumbed, in less than five minutes, to the intravenous injection of 0.5 c.c. of serum.

It was clear in this case, and abundantly confirmed later, that the peripheral application of serum not only sensitized in a peculiar way the peripheral respiratory epithelium but produced general sensitization as well. These results were to have been anticipated from the findings of other observers who used different methods. General body sensitization develops gradually during a course of local treatment with serum and apparently is preceded by the acquisition of local anaphylaxis. An effort was made to trace the development of the reactions of local and general anaphylaxis.

20. Rosenau and Anderson: Bull. Hyg. Lab., U. S. P. H. S., 1906, No. 29, p. 35.

EXPERIMENT 20.—Two normal male pigs, weighing 360 gm. each, October 11 were made to inhale, with intermissions, for about ten minutes the vapor of a 50 per cent. dilution of serum. There was slight temporary dyspnea, undoubtedly mechanical in origin and due to the long exposure to the vapor (the usual treatment occupied three minutes or less and the specific reaction commonly occurred within a minute or two). October 26, fifteen days later, the pigs having increased in weight, a similar less-prolonged treatment was given. There was no specific reaction, although Pig 1 breathed hard for a while ten minutes later. October 29, three days after the second and eighteen after the first inhalation, Pig 2 received, without ether, 0.5 c.c. of serum intravenously. There was no reaction. November 12 both pigs were again sprayed with serum of 50 per cent. dilution. Almost at once Pig 1, which had not received the intravenous injection, was seized with violent, rapid panting. This passed off in about fifteen minutes. Three days later in the morning this pig was found dead. I cannot account for the fatality except as a result of the treatment combined possibly with low resistance to the cool air of the night. Necropsy showed the olfactory lobes apparently much softened. The lungs were collapsed and congested but without hemorrhagic areas. Pig. 2, which had received intravenous injection October 29, received four serum-inhalations from November 15 to November 20. No dyspnea was caused by either treatment. This animal was given injection of serum by the vein November 23, and died with violent convulsive and bucking seizures.

These observations show that a single inhalation of serum by a normal guinea-pig provokes, when repeated fifteen days later, hardly any perceptible anaphylactic response. A third spraying, performed seventeen days after the last, produces a well-marked reaction. Intravenous injection of serum during these observations may have prevented, it certainly did not exaggerate, the reactions. It was found, as might have been expected, that guinea-pigs treated in precisely the same manner showed more or less individual differences in behavior. In contrast with the results of the last series of experiments, the following observation shows that possibly response to the local application of the antigen may be intensified by previous general sensitization.

EXPERIMENT 21.—Male, weight 570 gm. Olfactory lobes removed August 26 and the space left filled with melted paraffin. Sensitized subcutaneously with 0.33 c.c. of serum August 29. Received a prolonged inhalation of serum spray September 25, followed by no specific reaction. Inhalation was given again October 10, fifteen days later. A violent rapid dyspnea, which had not disappeared after an hour, immediately resulted. The treatment was repeated three times on alternate days, with identical response except that the latent period of reaction increased to a minute or two.

This experiment demonstrates again that response to the inhalation does not demand an intact olfactory apparatus.

I will now review rapidly the history of eighteen guinea-pigs, including individuals which were subcutaneously sensitized or normal and with olfactory lobes intact or removed. Half were treated primarily with instillations of serum into the nose and half with serum inhalations. Each group was again subdivided into two parts; the animals of one subgroup had serum dropped into the nose for four times on alternate days and those of the other for four times every fourth day.

The result was more or less active reaction with minor symptoms such as have already been described. After a resting interval of twelve days by the first group and of six days by the second group, which followed the last treatment in each, all of the nine "instillation" pigs were again given serum by the nose. To the local symptoms of nasal irritation and salivary secretion in certain animals was now added a more general reaction marked by more or less obvious dyspnea together with rattling, apparently bronchial râles. Of the two pigs in which the latter symptoms appeared, both had been subcutaneously sensitized and one had lost its olfactory lobes. Both râles and dyspnea were manifested only by animals which had received serum by the nose every fourth day. It is worth noting that when the group of animals treated in four sittings by serum dropped into the nose were given a like treatment eighteen days after the first dosage, the anaphylactic response was much more marked in animals which had received the preliminary instillations every *fourth* day.

In a parallel series of observations carried out with spray-inhalations, the reverse was noted; the strongest reactions occurred in animals receiving the four initial inhalations on *alternate* days. Five of the "instillation pigs" were given inhalations of serum on the day following the treatment which had produced so marked a reaction. The result was the production of a marked specific dyspnea in two of these animals. It is worth noting that of two pigs which had received no serum subcutaneously and from which the olfactory lobes had been removed, one responded by dyspnea to the spray and one did not. Of the two pigs not operated on, likewise without subcutaneous serum, neither responded. This experiment shows again that local sensitization of one part of the respiratory tract involves sensitization of the rest, though, as will be pointed out presently, there are marked local signs which distinguish the dyspnea produced by inhalation of serum in spray from that caused by its instillation in the nose.

Rapid dyspnea was the one sign of anaphylaxis observed in the inhalation experiments. It could be produced in nearly all animals, but some individuals responded much more readily than others. One animal which was made dyspneic by the inhalation, failed to react when sprayed daily, until the eighth day, when it responded again. In general, the daily administration of inhalations produced no response, though one pig which had received its first inhalation about two weeks previously responded each time to inhalation given every forty-eight hours. Of four animals which had received four sprayings on alternate days, from October 10 to 16, only one had shown marked reaction. They received another inhalation October 26 and each responded strongly — in fact the dyspnea in two cases was of desperate urgency.

Of these animals only two had been sensitized with serum subcutaneously. One of them had been deprived of olfactory lobes.

Five pigs which had received four inhalations from October 10 to 22 were likewise treated with the spray, October 26, but all responded very feebly. These animals were again given inhalations, November 2, and the dyspneic response was decidedly more vigorous though still much less marked than in the group treated after a longer passive interval. These results suggest, of course, the similar findings when anaphylaxis is studied through subcutaneous injections given at close intervals.

In a previous experiment it was shown that guinea-pigs sensitized by serum dropped into the nose could be made dyspneic at once by subjecting them to a spray of serum. The converse of this experiment was now performed. Four pigs that had reacted to the spray by dyspnea were given serum by the nose. In one there was reaction with only minor symptoms, but in the other three after long latent periods, five to ten minutes, rattling râles developed, which seemed to emanate from both the bronchi and the nose; there was also more or less cough.

As already mentioned, there is a marked difference in the quality of the anaphylactic response to the serum spray and serum instillation. In animals subjected to the former there are no râles, however violent the dyspnea. In animals responding strongly to the instillation, râles, which may be very loud and lasting, may occur with a variable element of dyspnea. The difference depends, probably, on the volume of secretion excited; that is, when a pig sensitized by instillation responds to the spray it is with a dry dyspnea; when a pig sensitized by the spray responds to instillation of serum droplets it is with a moist dyspnea. In later observations it was found that, in the shock resulting from the toxic intravenous injection, the influence of the "local sign" of the site of sensitization still held good. A pig which had been sensitized by the spray breathed very fast in its anaphylactic throes but developed no râles. Others which had been sensitized by instillation developed râles with, usually, less rapid breathing under the same conditions.

All the animals employed in these experiments were finally submitted to the supreme test of intravenous injection of serum. By this means it was sought to determine to what degree sensitization of the body could be developed by the peripheral application of serum, if at all, and also whether such application could essentially modify sensitization already induced by serum injected under the skin. The intravenous injections were given from twenty-nine to thirty days after beginning of the local treatment and after general sensitization had certainly been secured. Of the twenty and more animals available, four survived the first intravenous injection of serum.

Of eleven pigs treated by nasal instillation of serum three survived the first "toxic" injection. One of these belonged to a group of three from which the olfactory nerves and lobes had been removed but which had received no serum subcutaneously. This animal had been given serum by the nose four times at intervals of four days, from October 9 to October 21, and again on October 27. The creature had manifested plain signs of sensitization. Treated with serum spray, October 28, it became violently dyspneic. It was given 0.25 c.c. of serum intravenously, November 10. The animal was badly shocked in a manner peculiarly different from the ordinary anaphylactic seizures, that is, there were no clonic convulsions but the respiration was exceedingly rapid and made audible by râles. After about an hour, when the animal had apparently recovered, it suddenly suffered a fleeting twisting convulsion of the body. Thirteen days later, November 23, this animal received a second intravenous injection of more than 0.5 c.c. of serum. Great shock resulted with, at the outset, no sign of convulsion except clonic twistings. The respiration was very rapid and bronchial and nasal râles were loud. Finally, after sixteen days, December 9, a third intravenous injection of about 0.75 c.c. of serum was given. There was no anaphylactic reaction but great muscular weakness. Slight clonic twitchings set in after several hours, the weakness increased and the animal died in about twenty hours. Animals of this class also differed from those in ordinary anaphylactic reaction in that they appeared to be conscious even while lying on the side in deepest shock.

Of the other two "instillation pigs" that survived intravenous injection, both belonged to a group of three normal animals which had neither been operated on nor received serum subcutaneously. Their local treatment was similar to that of the last animal described except that neither was sprayed. Both of these animals, especially the second, had manifested slight but apparently unmistakable anaphylactic reaction to serum dropped into the nose. The first of the two was given 0.33 c.c. of serum intravenously November 8. The creature remained phenomenally quiet throughout the injection. There was no reaction whatever. The injection was repeated with 0.5 c.c. of serum, November 24: result, violent convulsions and typical death. I am inclined to interpret this case as further evidence that local sensitization may be achieved without producing any demonstrable general sensitization. The second surviving pig of this lot had especially reacted to instillation of serum by abundant secretions of saliva, an apparent sign of sensitization. Given an intravenous injection of 0.5 c.c. of serum, November 23, there was no reaction whatever (the animal was a female far advanced in pregnancy). Sixteen days later, the animal having borne a litter of young a week previously, a second intravenous injection of nearly 0.5 c.c. of serum

was given. There was no reaction except for rapid, jerky breathing and a few râles. Special stress deserves to be laid on the fact that this animal had achieved its wonderful power of self-protection by a course of treatment which had provoked exceedingly slight signs of anaphylaxis.

The single "inhalation-pig" that survived an intravenous injection of serum belonged likewise to a group of three which had neither been subcutaneously sensitized nor operated on. This animal did not react to repeated inhalation treatments until several had been given; then the response was slight but positive as to anaphylaxis. November 8, 0.5 c.c. of serum was given intravenously. The reaction was moderate but distinct. At the outset respiration was deepened and there were slight convulsive movements. Then the breathing became very rapid, interrupted by occasional very deep breaths, and so continued for half an hour. There were no râles as in the instillation pigs. November 24, sixteen days later, the treatment was repeated. Deep, rapid respiration with strangling cough came on at once. The animal died in five minutes *without having exhibited respiratory râles or any convulsions.*

It will be noted that none of the animals which had been previously sensitized by the subcutaneous administration of serum survived the toxic injection. The conclusion is that general sensitization so produced is not essentially modified by the efficient antigen applied to the peripheral epithelium, although general sensitization is undoubtedly achieved in this way. That this non-interference of the serum, peripherally applied, with the previous general sensitization, is not dependent on the time intervals of its application, seems shown by the fact that six "instillation" and four "spray" pigs received a course of seven daily treatments extending to within three days of the toxic injection; but only one survived it: In other cases the interval between the last local treatment and the toxic injection extended to more than twelve days. It is clear also that the olfactory apparatus, as far as represented by the olfactory lobes and intracranial nerves, is not essential to the systemic effects of serum locally applied. In fact, the normal animals apparently resisted general sensitization in an active way except as to the secretion of saliva. Of a group of animals reacting in about the same way to local treatment, one might survive and the others succumb to the toxic injection.

There was nothing in the results obtained by me to suggest any change in the respiratory epithelium resembling the "phenomenon of Arthus" (increasingly morbid local changes following repeated subcutaneous injections), which has been carefully investigated by Nicolle.[21] This subject was not, however, definitely investigated.

21. Nicolle, M.: Ann. de l'Inst. Pasteur, 1907, xxi, 128.

The statements of Friedberger, Ishioka and Busson in regard to pneumonic changes in the lungs of sprayed animals were, to a degree, confirmed. Such lungs at necropsy frequently revealed air-free hemorrhagic areas of limited extent. The affected portions when removed would sink in water, but they were soft and not resistant as in pneumonic consolidation. The post mortem change was by no means constant among sprayed pigs and was also found in animals dying from toxic injections, which had not been sprayed at all.

POSSIBLE ATROPHY OF THE OLFACTORY EPITHELIUM

Because of its importance I venture mention of a theme on which investigation was started but abandoned early in this work. In animals deprived of their olfactory lobes and even in some under other and functional conditions, there seemed to be a well-marked atrophy of the olfactory mucous membrane as disclosed by cutting away the roof of the nasal cavities. The subject was pursued no further than to give a basis for special research.

THE EFFECT ON TUBERCULOUS GUINEA-PIGS OF TUBERCULIN APPLIED TO THE PERIPHERAL EPITHELIUM

It was, of course, a surface thought that some useful therapeutic purpose might be served by treating a systemic affection through the peripheral epithelium. Tuberculous guinea-pigs were the subjects and the old tuberculin of Koch was the remedy. Six young guinea-pigs were inoculated with what proved to be a very attenuated culture of tubercle bacilli, kindly furnished me by Dr. G. B. Webb of Colorado Springs, July 25. Three of the animals were inoculated in the peritoneum and three beneath the skin. Two were used as controls and four were treated. The treatment consisted in dropping into the mouth of two subjects, in such a way as to excite the maximum taste-sensations, or in instilling into the nose (two subjects) dilutions of old tuberculin usually of from 0.5 to 1 per cent. Treatment given was from every day to every fourth day. Treatments were begun more than six weeks before the inoculations and were continued for more than two months afterward. Superficial observation could detect no particular reaction to the treatments except the excitement of slight dyspnea by spraying (which was practiced later). One of the treated pigs had to be killed, October 23, because of a tuberculous swelling occluding the urethra. The other pigs all gained in weight and were sacrificed for specific purposes nearly four months after their inoculation with tubercle bacilli. Post-mortem examination left no manner of doubt as to the effect of treatment: The extent and intensity of the tuberculous processes were much greater in the treated animals than in the controls.

DISCUSSION AND SUMMARY

Certain facts recorded in this investigation seem to furnish evidence which must be considered in further discussions of the old problem of the physiological requirements in ventilation. The trend of scientific opinion has lately been to ascribe the deleterious properties of rebreathed air solely to the untoward physicial conditions of heat and humidity usually involved. Rosenau and Amoss[22] recently published experiments tending back to the old "chemical view" of the noxiousness of rebreathed air. Their work has been controverted in literature not available to me.

The observations, detailed above, on the desensitization of guinea-pigs when exposed to the smell of horses, seem to establish beyond question, if such proof were needed, that animal effluvia when inhaled can produce at once profound systemic disturbance. It is usually taken for granted that "ventilation" has the same biological import for all people. On the contrary, it may well be that people suffering from various ailments may find in rebreathed air noxal elements specifically related to their infections (as did my guinea-pigs in the stable). Such a conception, of course, vastly complicates the biological consideration of ventilation. For many years I have noticed in carrying out physical examinations of the sick that the subjects of pulmonary tuberculosis appeared especially prone to avoid, as far as possible, the inhalation of the examiner's breath; it has seemed to me an instinctive effort toward specific self-protection.

Evidence has been brought forward which indicates a peculiar immunological relation between the central nervous system, or at least the brain, and the body. The brain can be sensitized and desensitized an indefinite number of times without essentially changing the susceptibility of the body (Besredka records three desensitizations in one animal within a few days). This desensitization of the brain after horse-serum injections may be brought about by a number of different agencies,[23] the specific antigen, as used by Besredka, or something allied to it and contained in the effluvia of horses, or, as I have shown, by cutting the olfactory nerves, lacerating the brain tissue or even by setting up mechanical vibrations in it. Rapid desensitization of the brain is marked by a more or less profound nervous shock followed by a brief period of antianaphylaxis during which the animal is absolutely protected from the poisonous antigen. The nerve impulses producing this remarkable somatic change must operate on the living tissues. It appears probable that these nerve impulses cause the tissues, as it were, to open their receptors for the specific antibody in the blood.

22. Rosenau and Amoss: Jour. Med. Research, 1911, xxv, 35.

23. Kolle and Wassermann: Handbuch der pathogenen Mikroorganismen, ii, Part 2, p. 1086.

If this "allergin" is removed from the circulation an animal has become essentially normal as regards reactivity to that antigen. Fixed in the tissues the allergin combines with more or less antigen contained therein and we witness a degree of shock proportional thereto. It is significant that all observers seem to agree that the allergin diminishes greatly or disappears from the circulation during the phase of anti-anaphylaxis.[24] According to the conception presented here the allergin is released from the tissues after a longer or shorter interval and, when returned to the circulation, susceptibility to the antigen is restored.

The widely accepted view of Friedberger[25] that antianaphylaxis depends on the saturation of the anaphylactic antibody with the newly introduced antigen cannot explain the condition when caused by brain mutilation; nor does this author's conception of the two states of non-specific and specific antianaphylaxis clear the matter. In fact, Bessau[26] makes a strong stand against Friedberger's theory and concludes that antianaphylaxis results from a reaction of the organism through which its irritability is depressed as regards the anaphylactic poison. In the masterly and lucid monograph of the Vaughans[27] it is held that the important factor which determines the refractory state of the reinjected animal "is the changed relation between the amount of ferment and the substrate," that is, the small amount of antibody remaining in the blood of the antianaphylactic animal becomes functionally inoperative in the presence of a great excess of antigen. This view, also, fails to account for the facts I have detailed. Evidence has been developed to show that the irritability of the brain, using this term in a broad physiological sense, is to an extraordinary extent dependent on olfactory stimuli. The teleological interpretation of these coordinations is irresistible. An animal smells many agents to which his body is sensitized and which, if eaten, would act as poisons *per se*, but through the olfactory sense the brain is sufficiently stimulated by the odor of the antigen to discharge impulses which set up in the body a state of antianaphylaxis during which the intrinsically poisonous substance may be ingested with impunity. The reaction of my guinea-pigs in the stable gives substantial support to such a conception. The possible relations of these conceptions to human physiology, psychology and surgery are obvious. The experiments with local applications of serum to the nose or by inhalation of a spray show clearly that sensitization of the respiratory epithelium develops after a definite latent period whose length does

24. See the valuable paper by Anderson and Frost, Bull. Hyg. Lab., U. S. P. H. S., 1910, No. 64.

25. Friedberger: Ztschr. f. Immunitätsforsch., 1909, ii, 208.

26. Bessau, G.: Centralbl. f. Bakteriol., 1911, lx, 637.

27. Vaughan, V. C., Vaughan, V. C., Jr., and Vaughan, J. W.: Protein Split Products in Relation to Immunity and Disease, 1913, p. 312.

not appear to be influenced by previous general sensitization of the body.

In contrast with this relative independence of central and peripheral sensitizations it is found that sensitization of the nasal respiratory tract involves the pulmonary epithelium, and *vice versa,* though each reacts with its own "local sign." The local reactions of the animal inevitably suggest that local anaphylaxis is a state of active resistance against absorption of the antigen. Transferring this idea to bacterial focal infections in the interior of the body, we get a more reasonable explanation of the beneficial results which often follow the injection of antigenous bacterial bodies; these can come into intimate contact with tissues capable of antibody formation from which the bacteria of the infective foci are debarred. Finally, the proof is once more absolute that serum dropped into the nose or given by inhalation sensitizes the body irrespective of the olfactory apparatus. The obvious explanation of the fact is that the antigen is absorbed by the circulation, as is supposed to be the case when serum is introduced into the rectum.[28] But "obvious explanations" are especially dangerous. Unfortunately, I have not been able to show that such peripheral application of the antigen in any way influences the reactions of a subcutaneously sensitized animal to the toxic intravenous injection. This seems a surprising fact in view of the accepted teaching that repeated injections of sublethal doses of serum produce in an animal a resistance, if not full immunity. That some peculiar status is occupied by the phenomena induced by application of serum to the respiratory epithelium seems shown by the fact that in two cases unsensitized guinea-pigs, from one of which the olfactory lobes had been removed, were somatically sensitized by this means and that they thereafter withstood several toxic injections separated by intervals of about two weeks. Experimental analogies have been presented for the anaphylactic phenomena of hay-fever and bronchial asthma. It is shown that both a general and a local sensitization are to be considered, interdependent, but each having an autonomy.

CONCLUSIONS

Briefly summarized, the following conclusions may be drawn:

1. Guinea-pigs sensitized to horse-serum may be desensitized by residence in a horse-stable. It is probable that this reaction may be rapidly achieved through the olfactory sense or more slowly by absorption through the respiratory mucous membrane. The period of anti-anaphylaxis is of less than two days' duration. The converse of this,

28. Vaughan, V. C.: Vaughan, V. C., Jr., and Vaughan, J. W.: Protein Split Products, 1913, p. 259.

sensitization of normal guinea-pigs in stable air, I have not been able to achieve.

2. Removal of part of the brain of a sensitized guinea-pig produces much more severe shock than the same amount of injury in a normal animal. This shock is apt to be followed by a period of antianaphylaxis of short duration.

3. Section of the olfactory nerves within the cranial cavity preceding the brain operation by a day or more abolishes the tendency to shock. Such nerve section in the normal animal also seems to reduce the shock arising from definite brain injury.

4. Depression of the olfactory sense may be produced through cocain and the symptoms of poisoning with this drug resemble those of anaphylaxis.

5. The body as a whole may be sensitized through the peripheral application of horse-serum after a definite latent period. The time required to sensitize the peripheral epithelium has not been found to be materially altered by previous general sensitization. A normal animal sensitized by local treatment may resist large intravenous injections of serum repeated at intervals of two weeks.

6. Sensitization of one area of the respiratory epithelium involves sensitization of the rest, though the local signs of expression are different.

7. The evidence indicates that local anaphylaxis, induced through the periphery, precedes the development of general sensitization.

8. Tuberculous guinea-pigs were made worse when treated by instillations or sprays of old tuberculin.

Besides the expression already made regarding Dr. Powell, it is a pleasure to acknowledge my indebtedness to my colleagues, Dr. W. C. Mitchell and Dr. P. Hillkowitz, for many favors, and to Dr. Auer of the Rockefeller Institute for hints as to technic.

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METABOLISM IN DEMENTIA PRAECOX*

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The findings reported in this paper are the result of work suggested by Dr. H. D. Singer, director of the institute, and begun by A. F. Wussow in this laboratory. Pighini[1] and Statuti[2] claimed from their data that the metabolism of dementia praecox was not normal. Wussow[3] and Barnes[4] did not obtain results that confirmed the findings of Pighini and Statuti. Folin[5] found no evidence of an abnormal metabolism in dementia praecox.

TESTS

The plan of the tests was to determine the income and the outgo of nitrogen, sulphur, phosphorus, calcium and magnesium in cases of dementia praecox. Tests were made at various times of the year and various diets were used (Table 1).

The method of preparing and preserving the food used in these tests made it possible to have but one sample of food for analysis and to give the patient a constant diet. For example, when hamburger was used as a part of the diet, sufficient very finely ground hamburger was cooked and then again very finely ground and mixed. Weighed amounts sufficient for a meal were put in ice-cream boxes and stored in a room which was maintained below 0 C. (32 F.). In the preparation of a meal one package of hamburger was put into a bowl with a small amount of water, set in a steam oven and warmed. Coffee, when used, was prepared by making up a liter or more of a very concentrated solution of commercial soluble coffee. Ten cubic centimeters of this were sufficient to make two cups of coffee. This amount was put into a pitcher with 20 c.c. of condensed milk and when the meal was ready to take to the ward, a couple of cups of boiling distilled water were added to each pitcher.

There were many difficulties to be overcome in doing metabolism work in cases of dementia praecox. One of the difficulties was with the feces. Cases of chronic or temporary constipation were frequently met.

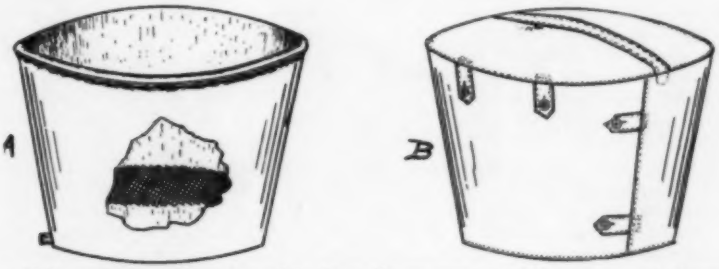
* From the Illinois State Psychopathic Institute.
* Submitted for publication Feb. 16, 1914.

1. Pighini: Arch. Neurol. and Psychiat., 1909, iv, 220.
2. Statuti: Am. Jour. Insanity, 1910, lxvii, 299.
3. Wussow: Institution Quarterly of Illinois, March 31, 1913, p. 71.
4. Barnes: Am. Jour. Insanity, 1909, lx, 591.
5. Folin, quoted by Wussow: The Institution Quarterly of Illinois, March 31, 1913.

TABLE 1.—Daily Diet in Metabolism Tests in Five Cases of Dementia Praecox

Patient	Preliminary Test	Test Proper	Breakfast	Dinner	Supper
G. G.	Sept. 30-Oct. 4	Oct. 4-10	Graham crackers, peanut butter, butterine, agar.	Same as breakfast.	Same as breakfast.
A. S.	Dec. 13-14	Dec. 14-20	Rice (boiled with milk), jelly (containing English walnuts), sugared agar.	Omelet (of ham, egg, cheese and milk), sugared agar.	Same as breakfast.
M. P.	Feb. 4-10	Feb. 10-15	(Ham - egg - cheese - milk) - omelet, graham crackers, peanut butter, butterine, agar, milk.	Same as breakfast.	Same as breakfast.
S. K.	Aug. 16-21	Aug. 21-29	Soda crackers, peanut butter, agar sauce.	Soda crackers, hamburger, agar sauce.	Graham crackers, peanut butter, agar sauce.
F. L.	Nov. 13-27	Nov. 27-Dec. 4	Coffee, condensed milk, egg omelet, graham crackers, agar sauce.	Coffee, condensed milk, hamburger, graham crackers, agar sauce.	Coffee, condensed milk, peanut butter, crackers, agar sauce.

A clear demarcation of the feces was many times prevented by soft or scanty stools. To overcome constipation, to insure frequent and copious bowel movements and to obtain a sharp marking of feces, agar was fed in all of the described tests. It takes considerable determination for a normal person to eat 10 gm. of shredded agar three times a day for two weeks, and consequently trouble was encountered in attempting to feed such an amount to disinterested insane persons. Patient G. G. was induced to eat 7 gm. of ground shredded agar at each meal when it was mixed with peanut butter and butterine, and spread on crackers. A number of failures followed the attempts to feed agar in this way to other patients. Patient A. S. took the agar when it was ground and coated with crystal sugar. The most palatable form of agar which has yet been prepared by us and which was used in the tests on Patients S. K. and F. L., was made by passing shredded agar through a sausage-grinder, moistening it with water and mixing with ground apple-sauce. This leaves the small pieces of agar tender enough to take away the straw-like consistency.



Urine and feces collector used in dementia praecox metabolism tests. *A* shows galvanized shelf which holds the feces, allowing urine to pass through; *B*, collector in canvas case.

Much difficulty was at first encountered in the collection of urine and feces separately or at all. All tests were made on women patients. Many refused to use either bed-pan or urinal, or both. If the patient did not object to using the bed-pan and urinal, often the collections were spoiled by the passage of both urine and feces at once into the bed-pan. These difficulties were all overcome by the construction of a collector, which is shown in the illustration.

Immediately after the passage of the excretions, the urine was drawn off into a bottle and the collector put into the canvas case and brought to the laboratory. Carmin was used as feces marker in obtaining the feces resulting from the test periods of feeding. The difficulty of getting the patient's bladder completely emptied at the end of each day was not overcome.

The patient in each case was kept out of bed all day and taken daily for a walk of about two miles in the open air. All of the cases were examples of dementia praecox. Patients G. G., A. S. and F. L. were of the chronic type. Patients S. K. and M. P. were of the subsiding acute type.

I am indebted to Dr. H. D. Singer and Dr. S. N. Clark for the following summaries of the cases.

REPORT OF CASES

CHRONIC STAGE OF HEBEPHRENIC DEMENTIA PRAECOX

CASE 1.—Patient F. L. has been in the Kankakee State Hosptal since January, 1904, and but little information is obtainable concerning her early life and the onset of the mental disorder. Her mother had been "slightly insane" for from fifteen to eighteen years. The patient was thought normal, married and had two children. In 1901, at the age of 32, she became depressed over the death of a child and following this became gradually more restless, excited and abusive and was treated at this hospital from August to December of that year. She was readmitted in January, 1904, and when received at the hospital was clear mentally, restless, irritable, experienced sense-falsifications with occasional violent reaction, and felt herself under outside influence. This early period of episodic restlessness, excitement and irritability apparently continued until 1907, when it was noted for the first time that she had become demented. She has since been indifferent, inactive and careless and untidy in appearance, with occasional episodes of noisy restlessness lasting a few days or weeks. She appears smiling, occasionally laughs and shows some stereotyped mannerisms such as suddenly pulling up her clothing. The metabolism test was performed in November and December, 1913, while patient was in a period of complete quiescence.

CASE 2.—Patient G. G., aged 25, was admitted to the Kankakee State Hospital on Aug. 22, 1912, with but scanty anamnesis. The mental picture had persisted with but little change for at least four years at that time. It was characterized by a general lack of interest in her surroundings with occasional outbursts of unexplained irritability, neglect of personal appearance and occasional uncleanliness. It was impossible to employ her steadily and for the most part she sat smiling to herself or wandered slowly and aimlessly. She appeared to be quite clear and to recognize her surroundings, but gave no connected information. Speech and actions were desultory with many oddities and stereotypies. There were no evidences of disease of the body organs. When admitted, the patient showed hemic murmur, but it has since disappeared. At the time of the test the picture was as described above and there has been no material alteration since.

CASE 3.—Patient A. S. was admitted to the Kankakee State Hospital, Dec. 9, 1910, aged 23. Very little is known of her early life, but she was considered an average child and graduated from common school at 14. She then went to work in a factory, but about two years later quietly gave it up and remained more or less indifferently at home. She became more and more careless and indifferent, developed certain peculiarities, such as touching various objects, putting odds and ends in her mouth, wandering away from home, etc., and probably experienced some sense falsifications, but without acute reactions. When admitted presented some physical signs suggestive of a tuberculous process in the lung, but there have been no cough, fever or loss of weight to suggest an active condition. The mental picture has been one of marked absence of spontaneity, a smiling compliance with all requests, indifference to personal appearance and cleanliness, and to the situation, in spite of full clearness. Answers to questions show much desultoriness of thought with some *vorbeireden*. Except for a

period of a few weeks beginning in February, 1911, when she became semistuporous, had to be spoon-fed and was more or less inaccessible though without rigidity of muscles, the picture outlined above has persisted unchanged. The metabolism test was made in December, 1912, while the patient was in this stationary condition.

SUBSIDING STAGE OF ACUTE KATATONIC DEMENTIA PRAECOX

CASE 4.—Patient M. P. was admitted to the Kankakee State Hospital, Dec. 28, 1912. Her mother has suffered from some psychosis, probably dementia praecox, since 1907. The patient was born in 1882 and is described as bright intellectually, with special interest in music, but always very nervous, self-conscious and irritable. She had no friends or confidants, was much alone and professed a hatred for male society. During the summer of 1912 she became depressed and later more restless with many odd acts and increasing inaccessibility. She also experienced auditory hallucinations. In December, 1912, she passed into a state of mutism and complete inactivity with some waxy rigidity of limbs, and was in this condition when admitted. The metabolism studies were undertaken in February, 1913, during this phase of katatonic stupor. During the earlier part of the tests she remained entirely mute and inaccessible, but towards the end of the two weeks she began to be slightly more active and to obey simple requests. The improvement continued subsequently but the patient has remained stilted and affected in speech and act, more or less indifferent to her surroundings, her friends, and her personal cleanliness, with outbursts of unmotived restless irritability and even violence, which occasionally interrupt a usual appearance of smiling indifference.

CASE 5.—Patient S. K. was admitted to the Kankakee State Hospital, July 25, 1913, aged 20. This patient is Bohemian and it has been impossible to obtain much anamnesis. She is described as an unusually bright child with no previous illness. In July, 1913, she became depressed and was admitted in a condition of marked katatonic stupor with absolute mutism and entire lack of spontaneous movement, with often some waxy rigidity and command automatism. Food was swallowed when placed in the mouth, but the patient paid no attention to flies or to other stimuli. August 1 she spoke for the first time since admission, but did not speak again before the 15th. The metabolism test was begun August 16 and continued until August 29, by which time it was possible to obtain a few answers to questions. She has since improved steadily and was allowed to return to her friends Nov. 16, 1913, having gained 23 pounds, though she still showed a lack of spontaneity with some vacuity and a failure to grasp the fact and nature of her illness. Conversation with her was very difficult owing to language differences.

Analyses of a composite of the daily food, finely ground and mixed, were made for nitrogen, sulphur, phosphorus, calcium and magnesium. The urine for the entire period was well mixed and analyses made of the mixture for the different forms of nitrogen and sulphur, in addition to the analyses made on the food. A composite of the total feces of the period was made in each case, and the same analyses made as on the food. The methods used are the following: Kjeldahl method for nitrogen, Folin's sodium-peroxid and Benedict's methods for total sulphur, Folin's methods for total sulphates and inorganic sulphates in urine, McCrudden's method for calcium and magnesium, Benedict's and Gephart's method for urea, Folin's method for ammonia, Folin-Shaffer method for uric acid and Folin's colorimetric method for creatinin. The official method of the Association of Official Agricultural Chemists was used to

determine the phosphorus after its oxidation. The oxidation in the case of the urine was with aqua regia, and in the case of foods and feces by ignition followed by digestion with concentrated nitric acid for four hours.

The accompanying tables contain the results of the five tests.

TABLE 2.—Nitrogen Expressed in Grams Per Day

Type	Patient	Length of Test Days	Food	Urine	Feces	Balance
Chronic..	F. L.	7	19.0230	7.6966	1.1616	10.1648+
	G. G.	6	18.2122	10.5703	1.5340	6.1078+
	A. S.	6	12.5496	11.8703	1.9313	1.2520—
Subsiding, acute..	M. P.	4	16.0290	7.2058	0.7714	8.0518+
	S. K.	8	10.4437	7.1181	0.9619	2.3637+

TABLE 3.—Distribution of Urinary Nitrogen Expressed in Grams Per Day

Type	Patient	Nitrogen Total	Urea	Ammonia	Creatinin	Uric Acid
Chronic..	F. L.	7.6966	6.8366	0.1514	0.3674	0.1433
	G. G.	10.5703	9.5321	0.2328	0.1093	0.0722
	A. S.	11.8703	10.5489	0.4349	0.2489	0.0423
Subsiding, acute..	M. P.	7.2058	5.3741	0.4426	0.1245	0.1035
	S. K.	7.1181	5.8038†	1.0097	0.3382	0.0738

TABLE 4.—Distribution of Urinary Nitrogen Expressed in Percentage of Total Nitrogen

Type	Patient	Nitrogen Total	Urea	Ammonia	Creatinin	Uric Acid
Chronic..	F. L.	100	88.87	1.97	4.78	1.86
	G. G.	100	90.17	2.00	1.03	0.68
	A. S.	100	88.82	3.66	2.09	0.37
Subsiding, acute..	M. P.	100	74.70	6.15	1.73	1.44
	S. K.	100	81.83†	14.19	4.78	1.04

† Determination was made on urine several days old; therefore the results are probably high.

TABLE 5.—Excretion of Sulphur Expressed in Grams Per Day

Type	Patient	Length of Test Days	Food	Urine	Feces	Balance
Chronic..	L. F.	7	1.9620	0.7155	0.4395	0.8070+
	G. G.	6	1.7216	0.3143	0.4378	0.9695+
	A. S.	6	1.0174	0.7691	0.4210	0.1727—
Subsiding, acute..	M. P.	4	2.1645	0.9768	0.5282	0.6895+
	S. K.	8	0.9756	0.5930	0.5233	0.1407—

TABLE 6.—Distribution of Urinary Sulphur Expressed in Grams Per Day

Type	Patient	Sulphur Total	Total Sulphate	Inorganic Sulphate	Neutral	Ethereal Sulphate
Chronic..	F. L.	0.7155	0.6321	0.5469	0.0834	0.0852
	G. G.	0.3142	0.2293	0.2756	0.0847	
	A. S.	0.7691	0.4550	0.4421	0.3140	0.0129
Subsiding, acute..	M. P.	0.9768	0.2858	0.2936	0.6910	
	S. K.	0.5930	0.4631	0.4291	0.1299	0.0340

TABLE 7.—Distribution of Urinary Sulphur Expressed in Per Cent. of Total Sulphur

Type	Patient	Total	Total Sulphate	Inorganic Sulphate	Neutral	Ethereal Sulphate
		Sulphur				
Chronic..	F. L.	100	88.30	76.30	11.64	11.90
	G. G.	100	72.99	87.72	27.02	
	A. S.	100	59.15	57.47	40.82	1.68
Subsiding, acute..	M. P.	100	29.26	30.06	70.76	
	S. K.	100	78.12	72.39	21.91	6.08

TABLE 8.—Phosphorus Expressed in Grams Per Day

Type	Patient	Length of Test Days	Food	Urine	Feces	Balance
Chronic..	F. L.	7	1.2825	0.4111	0.3798	0.4916+
	G. G.	6	2.2200	0.5369	1.1810	0.5021+
	A. S.	6	1.2960	0.7193	0.5676	0.0091+
Subsiding, acute..	M. P.	4	1.3552	.....	0.2474	..
	S. K.	8	0.7625	0.3393	0.6347	0.2115—

TABLE 9.—Calcium Expressed in Grams Per Day

Type	Patient	Length of Test Days	Food	Urine	Feces	Balance
Chronic..	F. L.	7	1.1003	0.2731	0.6978	0.1294+
	G. G.	6	2.4045	0.2272	1.8988	0.2785+
	A. S.	6	1.3068	0.2794	0.8368	0.1906+
Subsiding, acute..	M. P.	4	1.0960	0.5954	0.3032	0.1974+
	S. K.	8	0.6699	0.2472	0.4314	0.0087—

TABLE 10.—Magnesium Expressed in Grams Per Day

Type	Patient	Length of Test Days	Food	Urine	Feces	Balance
Chronic..	F. L.	7	0.6789	0.1281	0.2875	0.2633+
	G. G.	6	0.6313	0.1002	0.4931	0.0380+
	A. S.	6	0.3521	0.1031	0.2594	0.0104—
Subsiding, acute..	M. P.	4	0.4841	0.1161	0.1635	0.2045+
	S. K.	8	0.5356	0.0872	0.3302	0.1182+

TABLE 11.—Distribution of Various Elements Expressed in Relation to Nitrogen Valued at 100

Type	Patient	Food	Urine	Feces	Urine and Feces	Balance
				Sulphur		
Chronic..	F. L.	10.32	9.29	37.82	13.04	7.94+
	G. G.	9.45	2.97	28.54	6.21	15.87+
	A. S.	8.11	6.48	21.80	8.62	13.79+
Subsiding, acute..	M. P.	13.50	13.56	68.45	18.87	8.56+
	S. K.	9.34	8.33	54.42	13.82	5.95—
				Phosphorus		
Chronic..	F. L.	6.73	5.34	32.68	8.93	4.84+
	G. G.	12.19	5.08	76.78	14.20	8.22+
	A. S.	10.32	6.06	29.40	9.32	0.73—
Subsiding, acute..	M. P.	8.45	.....	32.06	.....	
	S. K.	7.30	4.77	66.01	12.06	8.95—
				Calcium		
Chronic..	F. L.	5.78	3.55	60.05	10.96	1.27+
	G. G.	13.20	2.15	123.78	17.57	4.56+
	A. S.	10.41	2.35	43.34	8.08	15.23—
Subsiding, acute..	M. P.	6.84	8.26	39.29	11.27	2.45+
	S. K.	6.42	3.47	44.86	8.40	0.37—
				Magnesium		
Chronic..	F. L.	3.57	1.66	24.74	4.69	2.59+
	G. G.	34.66	0.95	32.14	4.90	0.62+
	A. S.	2.80	0.87	13.43	2.62	0.83+
Subsiding, acute..	M. P.	3.02	1.61	21.19	3.50	2.54+
	S. K.	5.13	1.22	34.34	5.17	4.99+

DISCUSSION OF RESULTS

Total Nitrogen.—Table 2 indicates nothing of particular interest concerning the nitrogen distribution. With an intake of 12.5 gm. of nitrogen as in the case of Patient A. S., a negative balance is hardly to be expected. The excessive amount of nitrogen in the urine, which was responsible for this negative balance is more or less constant for this patient as shown in a previous test.[6]

Nitrogen Partition, Urea Nitrogen.—The urea nitrogen in the several cases varied from 5.37 to 9.53 gm. daily. These values are within the range of the normal urea output, but nearer the minimum than the maximum limit. When the patients are grouped according to the two classes of dementia praecox, one is immediately struck with the fact that, as far as the urea nitrogen is concerned, each class seemed to have a field of variation of its own. The chronic cases excreted from 6.84 to 10.55 and the acute cases from 5.37 to 5.80 gm. of urea nitrogen. The same class difference is indicated in Table 4 in which the urea nitrogen is expressed in per cent. of the total urinary nitrogen. In the chronic cases it is from 88.8 to 90.2, while in the acute cases it is from 74.7 to 81.8 per cent.

Folin[7] shows that the urea output is governed to some extent by the protein intake. Folin fed 118 gm. of protein and found a urea-nitrogen output of 14.7 gm. which constituted 87.5 per cent. of the total urinary nitrogen. When he fed 6 gm. of protein, the urea-nitrogen excretion decreased to 2.2 gm., making up only 61.7 per cent. of the total urinary nitrogen. The question now arises as to whether or not in our cases the class differences in the output of urea nitrogen in grams and in per cent. were not due to a difference in the nitrogen intake. If this were true, there should be no constant difference between the two classes of patients in the number of grams of urea nitrogen excreted per gram of nitrogen ingested, and in the per cent. of urea nitrogen making up the total urinary nitrogen per gram of nitrogen ingested. That this is the case in both of the relations is shown by the following figures for Patients F. L., G. G., A. S., M. P. and S. K., respectively, 0.359, 0.523, 0.841, 0.335 and 0.556 gm. of urea nitrogen per gram of nitrogen ingested, and 4.67, 4.95, 7.08, 4.66 and 7.84 per cent. of total urinary nitrogen in the form of urea nitrogen per gram of the total nitrogen intake. Since these figures do not show any definite distinction between the two classes of patients, and since it is well known that the urea nitrogen is governed to some extent by the nitrogen intake, we must conclude that in this test

6. Ross, Ellison L.: Some Forms of Urinary Nitrogen Affected by the Administration of Desiccated Thyroid to Dementia Praecox Patients, THE ARCHIVES INT. MED., 1913, xii, 746.

7. Folin: Quoted by Lusk in Science of Nutrition, p. 138.

the differences in urea excretions were due to the differences in the nitrogen intake.

Ammonia Nitrogen.—Normally ammonia nitrogen varies from about 0.2 to 1 gm. per day. The amount varies in normal individuals with the diet, depending on the amount of protein ingested and on the amount of acid-forming constituents and alkali metals of the diet. In pathological cases excessive ammonia output indicates an increased acid formation within the organism. Von Noorden[8] states that when the ammonia nitrogen of the urine does not exceed from 8 to 10 per cent. of the total nitrogen intake it cannot be said to be abnormal.

In this test the ammonia nitrogen varied from 0.15 to 1.01 gm. per day, entirely within the normal so far as the absolute quantities are concerned. The difference between the two classes of dementia praecox in this is distinct, but not great enough to prohibit the explanation of the difference on the grounds of differences in the character of the food.

In Table 4 the ammonia nitrogen is expressed in per cent. of the total nitrogen of the urine. Expressed in this way, ammonia nitrogen makes up from 2.0 to 14.2 per cent. of the total amount in the urine. Von Noorden[8] considers this manner of expressing the amount of ammonia nitrogen of no value. That this is true is indicated by Folin's[7] test, cited before, in which 118 gm. were fed with an output of 16.8 gm. of urinary nitrogen and 0.49 gm. of ammonia nitrogen, making up 3.0 per cent. of the urinary nitrogen, whereas when 6 gm. of protein were fed, 3.6 gm. of nitrogen appeared in the urine, of which 0.42 gm., or 11.3 per cent. was ammonia nitrogen. In these two tests there were about equal quantities of ammonia nitrogen expressed in grams, but nearly four times as much in the second test as in the first, if measured by the per cent. of the total urinary nitrogen, which made up from 60 to 90 per cent. of the variable urea nitrogen. It is an interesting fact, however, that in the acute cases a greater per cent. of nitrogen in the form of ammonia nitrogen was excreted than in the chronic cases.

If the ammonia nitrogen were expressed in per cent. of the total nitrogen ingested we would have for Patients F. L., G. G., A. S., M. P., and S. K., the following values: 0.80, 1.28, 3.46, 2.76, and 9.71 per cent. These figures show a tendency in the acute cases to the excretion of the greater amount of ammonia nitrogen. The average of the values for the acute cases, 6.23 per cent., is larger than the average of any two cases in the chronic group and considerably larger than the average of all of those in the chronic group, 1.85 per cent. According to Von Noorden this variation not being outside of from 8 to 10 per cent., could not be said to be abnormal.

8. Von Noorden: Metabolism and Practical Medicine, I, 106.

Creatinin and Uric Acid Nitrogen.—No distinction can be detected between the two classes of patients by their output of creatinin and uric acid nitrogen. The amounts of creatinin and uric acid nitrogen are within the range of normal persons, according to Schaffer,[9] Hawk,[10] Neubauer, P. Munk and C. Voit,[11] Van Noorden[12] and Folin.[13]

Total Sulphur.—Table 5 indicates the distribution of the total sulphur. The intake varied from 0.97 to 2.16 gm. per day. The urinary sulphur had a greater variation, from 0.31 to 0.98 gm. per day. The fecal sulphur was remarkably constant for the two classes of patients. In the chronic class it was from 0.42 to 0.44 gm. and in the acute class from 0.52 to 0.53 gm. per day. Undoubtedly the greater part of this sulphur in the feces was in the indigestible agar, which contains about 1 per cent. of sulphur. The balances of sulphur are such as might be expected to accompany the nitrogen balances given in Table 2, except in the case of Patient S. K., who showed a positive nitrogen balance, but a negative sulphur balance. Although the character of the diet given in Table 1 is usually considered a well-balanced one, the analysis of the food and feces seems to indicate that the available sulphur occurred in an insufficient quantity in the diet of S. K.

Table 11 indicates the sulphur distribution relative to the total nitrogen distribution, the latter taken as a standard being evaluated as 100. The sulphur-nitrogen relation in the foods of the five patients varied considerably—from 8.11 to 13.50 per cent. This variation is undoubtedly due to the agar, which is poor in nitrogen and contains about 1 per cent. of sulphur.

The sulphur-nitrogen relation for the urines is interesting in the wide range of variation of from 2.97 to 13.56 per cent. The same large variation exists for the feces and for the total outgo. In the case of the urine, feces and total outgo, the sulphur-nitrogen relation of the two classes of patients materially differs. The average of all of the values or any two of the values for the chronic cases is less than the average for the acute cases. It therefore may be concluded from these tests that in the subsiding acute cases of dementia praecox there is a tendency to excrete more sulphur in proportion to nitrogen in both urine and feces than in the cases of chronic dementia praecox. Whether

9. Shaffer: Quoted by P. B. Hawk in Practical Physiological Chemistry, Ed. 3, p. 270.

10. Hawk: Quoted by P. B. Hawk in Practical Physiological Chemistry, Ed. 3, p. 266.

11. Neubauer, Munk P., and Voit, C.: Quoted by Von Noorden in Metabolism and Practical Medicine, i, p. 127.

12. Von Noorden: Metabolism and Practical Medicine, i, p. 115.

13. Folin: Quoted by Lusk in Science of Nutrition, pp. 138-364.

or not this tendency is outside of the limits of normal variation cannot be determined from the data available.

Sulphur Partition.—Total Sulphate-Sulphur.—The patients in this test excreted in the urine from 0.23 to 0.63 gm. of sulphur in the form of sulphates. These amounts made up from 29.26 to 88.30 per cent. of the total sulphur in the urine. While the total sulphate-sulphur of the urine is dependent on both diet and physical condition of the subject, Hawk[14] states that normally about 2.5 gm. of sulphuric acid or about 0.8 gm. of sulphur are excreted per day in the sulphate form. Folin[5] gives the total sulphate-sulphur output of two normal cases on a purin-free diet. The first case gave an average of 1.14 gm. or 94.2 per cent. of the total urinary sulphur. The second case gave 0.87 gm. or 86.9 per cent. of the total urinary sulphur. Mandel and Lusk[16] found that a normal subject, on a six-day test, excreted, for every 100 gm. of nitrogen ingested, an average of 6.24 gm. of sulphur in the form of sulphates. Our patients, taken in the order in which the cases are arranged in the tables, excreted, for every 100 gm. of nitrogen ingested, 3.32, 1.26, 3.62, 1.83 and 4.45, or an average of 2.90 gm. of sulphur in the form of sulphates. While the above comparisons, made with the total sulphate output in these cases of dementia praecox, have not been closely comparable for reasons of differences of diet, subjects and conditions of subjects, they have shown that the patients of our test did not excrete normal amounts of sulphate sulphur.

Inorganic Sulphates: Tables 6 and 7 show the amount of sulphur in the form of inorganic sulphates in the urine to have varied from 0.27 to 0.55 gm. per day, or from 30.06 to 87.72 per cent. of the total urinary sulphur. There is also a suggestion of a difference between the two classes of dementia praecox in the amounts of inorganic sulphates excreted. The average of the two lowest values in the chronic cases is 0.3588 gm., or 72.59 per cent. of the total urinary sulphur, and the average in all three cases is 0.4215 gm., or 73.83 per cent. of the total urinary sulphur, while the average in the subsiding acute cases is 0.361 gm., or 51.22 per cent. of the total. There is an overlapping of values in the two classes, so that the class difference must be considered small. As to the normality of the values as a whole for the inorganic sulphate sulphur output, there is little question. The only data at hand for comparison are those of Folin.[15] On a purin-free diet, Folin's patients averaged 1.028 gm., or 86.2 per cent., and 0.80 gm., or 78.7 per cent. of the total sulphur. According to these figures the chronic

14. Hawk: Practical Physiological Chemistry, Ed. 3, p. 291.

15. Folin: Quoted by Lusk in Science of Nutrition, p. 364.

16. Mandel and Lusk: Quoted by Von Noorden in Metabolism and Practical Medicine, iii, 613.

patient's output of inorganic sulphate would be nearly normal so far as the percentage of the total sulphur is concerned, but low in absolute quantities. Compared in the same way, in the acute cases the patients were below normal both in absolute weight of inorganic sulphate sulphur and in its relation to the total urinary sulphur.

Neutral-Sulphur.—The neutral-sulphur output of our patients is especially interesting in that it confirms Pighini's[1] findings. The absolute amount varies from 0.0834 to 0.6910 gm., making up from 11.64 to 70.76 per cent. of the total urinary sulphur. While there is some overlapping of values in the two classes of patients, there seems to be a distinct tendency in the acute cases to the excretion of the greater amount of neutral-sulphur. The average of the two highest values in the chronic class is 0.1883 gm., making up 33.92 per cent. of the total sulphur, and the average of all three chronic cases 0.1607 gm., or 26.49 per cent. of the total sulphur of the urine, while the average output in the acute cases was 0.4104 gm., or 46.33 per cent. of the total urinary sulphur.

Sherman[17] states that normally from 5 to 15 per cent. of the total urinary sulphur is neutral-sulphur. Von Noorden finds that the neutral sulphur normally makes up from 14 to 25 per cent. of the total urinary sulphur. Folin's cases averaged 5.8 and 13.1 per cent., or 0.14 and 0.10 gm., of neutral-sulphur. These figures indicate that in the chronic cases an amount of neutral-sulphur very close to the limit of normal subjects was excreted, and that the acute cases were quite abnormal in this respect.

Ethereal Sulphates.—The data on the ethereal sulphate-sulphur output are not satisfactory or sufficient to be of any considerable importance. The tendency seems to be toward a low percentage for this form of sulphur.

Phosphorus.—Table 8 gives the distribution of phosphorus in the food, urine, feces, and the balance. The intake varied from 0.76 to 2.22 gm. Sherman[18] states that in normal subjects about 1.2 gm. are necessary for a phosphorus balance to result, but that a phosphorus balance has been obtained on as low as 0.90 gm. per day. From these facts it is evident that Patient S. K. did not receive sufficient phosphorus for a normal person. Therefore the negative phosphorus balance in this patient was to have been expected. In the urine the phosphorus seems to have been more normal in all cases. Von Noorden[19] states that normally the ratio $P_2O_5 : N = 1 : 7$. In our cases the ratio varies from 1:7.1 to 1:9.1.

17. Sherman: Chemistry of Food and Nutrition, p. 268.
18. Sherman: Chemistry of Food and Nutrition, p. 278.
19. Von Noorden: Metabolism and Practical Medicine, iii, 817.

Calcium.—The calcium distribution shown in Table 9 is relatively a normal one. The intake varied from 0.6699 to 2.4045 gm. per day. Sherman[20] states that the normal body requires from 0.7 to 1.0 gm. of CaO, or expressed in terms of Ca, from 0.5 to 0.7 gm. per day. The calcium intake of Patient S. K. was the only one which approached the minimum limit. That it was her smallest calcium requirement is shown by her very small negative balance. The amount of calcium in the urine varied from 0.2272 to 0.5954 gm., which, according to von Noorden,[21] is normal. No difference between the two classes of patients can be detected from the absolute amounts excreted or the amounts relative to the nitrogen given in Table 2.

Magnesium.—Renvall[22] asserts that man normally needs 0.45 gm. of magnesium per day. Langworthy[23] estimates the normal bodily requirement of magnesium to be from 0.18 to 0.30 gm. per day. Our patients received from 0.3521 to 0.6789 gm. per day. According to Langworthy's standard all patients received sufficient magnesium. According to Renvall's standard all but one of our patients had a sufficiency of this element and that patient had a small negative balance. The magnesium content of the urines was quite constant, varying from 0.0872 to 0.1281 gm. The feces content was quite variable because of the relative richness of the agar in the food. The balances show nothing of importance. The absolute amounts and the relative amounts of magnesium to the nitrogen in the urines, feces and balances do not show any differences between the two classes of dementia praecox.

SUMMARY

Five cases of dementia praecox, three of them in a chronic and two in a subsiding acute phase of the disease, were used in the metabolism tests. Over a period of from four to eight days the exact income and outgo of total nitrogen, different forms of nitrogen, total sulphur, different forms of sulphur, phosphorus, calcium and magnesium were determined.

The metabolism of total nitrogen was found to be within the limits of normal with the possible exception of one case.

The urea nitrogen of the urine was variable but remained normal.

The ammonia nitrogen of the urine varied within the limits found in normal cases. In the acute cases a tendency was exhibited to excrete more of this form of nitrogen than in the other class of patients.

20. Sherman: Chemistry of Food and Nutrition, p. 288.

21. Von Noorden: Metabolism and Practical Medicine, iii, 39.

22. Renvall: Quoted by von Noorden in Metabolism and Practical Medicine, i, 428.

23. Langworthy: Quoted by E. B. Forbes in Bull. Ohio Experiment Station, No. 201, p. 165.

The amounts of creatinin and uric acid nitrogen obtained in these cases of dementia praecox coincide with the normal.

The metabolism of total sulphur is considered normal, with the acute cases showing a greater sulphur excretion, both in urine and feces, than the other class of patients.

The total sulphate-sulphur output, according to the data on normal cases cited by authorities, was below normal.

The inorganic sulphate-sulphur of the urine, according to data on normal subjects, was below normal in absolute quantities in both classes of patients, and lowest in the case of the acute class. The amounts of this form of sulphur expressed in percentage of the total urinary sulphur, were about normal for the chronic class but below normal for the acute group.

The urinary neutral-sulphur was practically normal for the chronic cases, but much above normal in the subsiding acute cases.

The ethereal sulphate findings were negative.

As to the meaning of these positive findings on the sulphur metabolism there is little question. In the chronic cases there was normal total sulphur, practically normal neutral sulphur, below normal total sulphate and possibly below normal inorganic sulphate. In the acute cases we have a normal, but a maximum normal, excretion of total sulphur, subnormal total sulphate, subnormal inorganic sulphate and supernormal neutral sulphur. E. Salkowski and Rudenko[24] state the well-known fact that an increased neutral-sulphur output at the expense of the sulphates is an indication of reduced power of tissue oxidation. Therefore it must be concluded from the sulphur data that in the acute cases the patients had lost to a certain degree their normal powers of oxidation. Also this is suggested in the patients in the chronic class by the subnormal sulphate output. That a reduced power of oxidation should occur in cases of acute dementia praecox is not surprising, since seemingly every bodily activity is decreased. That indol-acetic acid is very frequently found in the urines of dementia praecox patients[8] is evidence of a diminished power of oxidation.

The findings on phosphorus, calcium and magnesium were negative.

CONCLUSIONS

In these cases of dementia praecox the data show the following facts:

1. The metabolism of nitrogen, phosphorus, calcium and magnesium was normal in both classes of patients.

24. Salkowski, E., and Rudenko: Quoted by von Noorden in Metabolism and Practical Medicine, ii, 355.

2. The metabolism of sulphur, except in the excessive total sulphate output and possibly the inorganic sulphate output, was normal for the chronic cases.

3. The metabolism of sulphur in the acute cases was abnormal in that the excretion of total sulphur was maximum normal, the total sulphate and inorganic sulphate output were subnormal and the neutral sulphur of the urine was above normal.

4. The abnormalities in the sulphur metabolism of the acute forms indicate a reduced power of metabolic oxidation. The fewer and less pronounced abnormalities of sulphur metabolism in the chronic group suggest a similar, though less extensive deficiency, than in the case of the acute group.

THE TITRATION OF WASSERMANN REAGENTS *

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As the Wassermann test is considered, and should be, a quantitative reaction, the correct titration of the reagents, especially complement and amboceptor, is of the utmost importance.

Wassermann,[1] in the original communication, and Noguchi,[2] as well as other writers on the subject, recommend the titration of complement and amboceptor without the addition of the other factors. That is, these two factors are mixed in varying quantities with a suspension of corpuscles. On incubation the smallest amount of the factor which, with a fixed quantity of the other, is required to produce complete hemolysis, is considered as one unit. For the actual test twice the unit is used.

This method of titration is open to objection. Noguchi has pointed out that within certain limits complement and amboceptor are interchangeable. Further, it is a recognized fact that on the addition of patients' serum (whether positive or negative) or antigen, more complement or amboceptor is required to produce hemolysis than when neither of these factors is added. This is overcome by doubling the units of complement and amboceptor.

The objection is that the worker does not know that twice the amount of complement and amboceptor which are required to produce hemolysis without serum and antigen will just produce hemolysis with them; that is, of course, if the serum is from a non-luetic patient, and the worker does not know that, when just a small amount of the complement present is bound by a slightly positive serum, enough complement may be left to produce complete hemolysis, and a weakly positive test be changed to a negative.

The following method of titration of complement and amboceptor has been adopted by me in my Wassermann work:

Actual test conditions are imposed on the factors throughout. The human hemolytic system is employed. The method of preparing the reagents has been described in a previous article.[3]

* Submitted for publication Dec. 8, 1913.

* From the Laboratory of Clinical Diagnosis, Medical Department, University of Arkansas.

1. Deutsch. med. Wchnschr., 1906, xxxii, 745.
2. Noguchi, H.: The Serum Diagnosis of Syphilis, Philadelphia, 1911.
3. Thompson, Loyd Oscar: A Modified Wassermann, THE ARCHIVES INT. MED., 1913, xi, 512.

The corpuscle suspension is a 2.5 per cent. solution of washed human erythrocytes. The complement is a 10 per cent. solution of guinea-pig serum, while the amboceptor is a solution of the serum of a rabbit, which has been immunized to human corpuscles, and diluted according to the titrated value.

The corpuscle suspension is, of course, fixed and when amboceptor is titrated, it is necessary first to titrate the complement to be used against a known amboceptor, or to fix the dose of complement arbitrarily. The latter is the usual method and I choose 0.5 c.c. as the most common unit.

Fifteen tubes are required for the titration of amboceptor and eight tubes for controls. The controls are the same as those used in the actual test. In each of the fifteen tubes is placed 0.1 c.c. of a known negative inactivated serum, 0.5 c.c. of complement, 1 unit of antigen (diluted so that 1 unit = 0.1 c.c.), and the amount of physiological salt solution required to bring the total volume up to 2.5 c.c., as indicated in Table 1.

TABLE 1.—TITRATION OF AMBOCEPTOR

Tube	Serum	Comp.	Ant.	NaCl	Amboceptor	Corp.	Total	Results
1	0.1	0.5	0.1	0.8	0.5 of 1:10,000	0.5	2.5	NH
2	0.1	0.5	0.1	0.7	0.6 of 1:10,000	0.5	2.5	NH
3	0.1	0.5	0.1	0.6	0.7 of 1:10,000	0.5	2.5	PH
4	0.1	0.5	0.1	0.5	0.8 of 1:10,000	0.5	2.5	PH
5	0.1	0.5	0.1	0.4	0.9 of 1:10,000	0.5	2.5	PH
6	0.1	0.5	0.1	1.2	0.1 of 1: 1,000	0.5	2.5	PH
7	0.1	0.5	0.1	1.1	0.2 of 1: 1,000	0.5	2.5	PH
8	0.1	0.5	0.1	1.0	0.3 of 1: 1,000	0.5	2.5	H
9	0.1	0.5	0.1	0.9	0.4 of 1: 1,000	0.5	2.5	H
10	0.1	0.5	0.1	0.8	0.5 of 1: 1,000	0.5	2.5	H
11	0.1	0.5	0.1	0.7	0.6 of 1: 1,000	0.5	2.5	H
12	0.1	0.5	0.1	0.6	0.7 of 1: 1,000	0.5	2.5	H
13	0.1	0.5	0.1	0.5	0.8 of 1: 1,000	0.5	2.5	H
14	0.1	0.5	0.1	0.4	0.9 of 1: 1,000	0.5	2.5	H
15	0.1	0.5	0.1	0.3	1.0 of 1: 1,000	0.5	2.5	H
16	0.0	0.5	0.0	0.5	1.0 of 1: 1,000	0.5	2.5	H
17	0.0	0.5	0.1	0.4	1.0 of 1: 1,000	0.5	2.5	H
18	0.0	0.5	0.1	1.4	0.0	0.5	2.5	NH
19	0.0	0.5	0.0	1.5	0.0	0.5	2.5	NH
20	0.0	0.0	0.1	0.9	1.0 of 1: 1,000	0.5	2.5	NH
21	0.0	0.0	0.1	1.9	0.0	0.5	2.5	NH
22	0.0	0.0	0.0	1.0	1.0 of 1:1,000	0.5	2.5	NH
23	0.0	0.0	0.0	2.0	0.0	0.5	2.5	NH

The tubes are now incubated one hour in the incubator or half an hour in the water-bath at 37 C., after which the amboceptor and corpuscles are added.

Tube 1 contains 0.5 c.c. of a 1:10,000 dilution of amboceptor and the amount is increased until Tube 15 contains 1 c.c. of a 1:1,000 dilution, or 0.01 c.c. of pure serum. The incubation is continued two hours in the incubator, or one hour in the water-bath at 37 C. At the end of this time the tube which contains the least amboceptor, which shows com-

plete hemolysis, is determined and this amount of amboceptor is considered as 1 unit.

The titration of complement is similar except that here the amboceptor is the fixed quantity and the tubes contain increasing amounts of complement, as indicated in Table 2.

TABLE 2.—TITRATION OF COMPLEMENT

Tube	Ser.	Comp.	Ant.	NaCl	Amb.	Corp.	Total	Results
1	0.1	0.4	0.1	1.3	0.1	0.5	2.5	PH
2	0.1	0.5	0.1	1.2	0.1	0.5	2.5	PH
3	0.1	0.6	0.1	1.1	0.1	0.5	2.5	PH
4	0.1	0.7	0.1	1.0	0.1	0.5	2.5	H
5	0.1	0.8	0.1	0.9	0.1	0.5	2.5	H
6	0.1	0.9	0.1	0.8	0.1	0.5	2.5	H
7	0.1	1.0	0.1	0.7	0.1	0.5	2.5	H
8	0.0	1.0	0.0	0.9	0.1	0.5	2.5	H
9	0.0	1.0	0.1	0.8	0.1	0.5	2.5	H
10	0.0	1.0	0.1	0.9	0.0	0.5	2.5	NH
11	0.0	1.0	0.0	1.0	0.0	0.5	2.5	NH
12	0.0	0.0	0.1	1.8	0.1	0.5	2.5	NH
13	0.0	0.0	0.1	1.9	0.0	0.5	2.5	NH
14	0.0	0.0	0.0	1.9	0.1	0.5	2.5	NH
15	0.0	0.0	0.0	2.0	0.0	0.5	2.5	NH

In the actual performance of the test, one unit each of complement and amboceptor, and only one, is used. This is all that is required, since it has been determined that, with these amounts, we have secured complete hemolysis with a known negative serum, and if, with an unknown serum, we get any inhibition of hemolysis, we know that something has bound at least part of the complement, which indicates a positive reaction.

In the titration of antigen the first and most important point is to determine whether or not the antigen is hemolytic in an amount considerably in excess of the antigenic unit. This is determined by adding to a corpuscle suspension gradually increasing amounts of the antigen, as indicated in Table 3.

TABLE 3.—TITRATION OF ANTIGEN (HEMOLYTIC EFFECT)

Tube	Ant.	Corp.	NaCl	Total	Results
1	0.5 of 1:100	0.5	1.5	2.5	NH
2	0.6 of 1:100	0.5	1.4	2.5	NH
3	0.7 of 1:100	0.5	1.3	2.5	NH
4	0.8 of 1:100	0.5	1.2	2.5	NH
5	0.9 of 1:100	0.5	1.1	2.5	NH
6	0.1 of 1: 10	0.5	1.9	2.5	NH
7	0.2 of 1: 10	0.5	1.8	2.5	NH
8	0.3 of 1: 10	0.5	1.7	2.5	NH
9	0.4 of 1: 10	0.5	1.6	2.5	NH
10	0.5 of 1: 10	0.5	1.5	2.5	NH
11	0.6 of 1: 10	0.5	1.4	2.5	NH
12	0.7 of 1: 10	0.5	1.3	2.5	NH
13	0.8 of 1: 10	0.5	1.2	2.5	NH
14	0.9 of 1: 10	0.5	1.1	2.5	NH
15	1.0 of 1: 10	0.5	1.0	2.5	NH
16	1.5 of 1: 10	0.5	0.5	2.5	PH
17	2.0 of 1: 10	0.5	0.0	2.5	H

Tube 1 contains 0.5 c.c. of a 1:100 dilution of antigen, while Tube 17 contains 2 c.c. of a 1:10 dilution, or 0.2 c.c. of pure antigen.

The tubes are now incubated for one hour in the water-bath at 37 C., and the one containing the largest amount of antigen in which there is no hemolysis is recorded.

Working with Dr. Gabby of the Psychopathic Institute at Kankakee, Ill., and Dr. Nichols of the Presbyterian Hospital, Chicago, I have shown that certain antigens, which have a marked hemolytic action when added to corpuscles alone, have this action inhibited when either human or guinea-pig serum is added in small quantity (0.1 c.c.).

The question naturally presents itself, whether or not such an antigen would be a safe one to use, since antigen is never used in a test without the presence of patients' serum and complement. I do not consider it safe, because if it is used, and the corpuscles are hemolyzed, it would not be known how much of the hemolysis is due to the amboceptor and complement, and how much to the antigen.

TABLE 4.—TITRATION OF ANTIGEN (ANTICOMPLEMENTARY VALUE)

Tube	Ser.	Comp.	Ant.	NaCl	Amb.	Corp.	Total	Results
1	0.1	0.5	0.5 of 1:100	0.8	0.1	0.5	2.5	H
2	0.1	0.5	0.6 of 1:100	0.7	0.1	0.5	2.5	H
3	0.1	0.5	0.7 of 1:100	0.6	0.1	0.5	2.5	H
4	0.1	0.5	0.8 of 1:100	0.5	0.1	0.5	2.5	H
5	0.1	0.5	0.9 of 1:100	0.4	0.1	0.5	2.5	H
6	0.1	0.5	0.1 of 1: 10	1.2	0.1	0.5	2.5	H
7	0.1	0.5	0.2 of 1: 10	1.1	0.1	0.5	2.5	H
8	0.1	0.5	0.3 of 1: 10	1.0	0.1	0.5	2.5	H
9	0.1	0.5	0.4 of 1: 10	0.9	0.1	0.5	2.5	H
10	0.1	0.5	0.5 of 1: 10	0.8	0.1	0.5	2.5	H
11	0.1	0.5	0.6 of 1: 10	0.7	0.1	0.5	2.5	H
12	0.1	0.5	0.7 of 1: 10	0.6	0.1	0.5	2.5	H
13	0.1	0.5	0.8 of 1: 10	0.5	0.1	0.5	2.5	H
14	0.1	0.5	0.9 of 1: 10	0.4	0.1	0.5	2.5	H
15	0.1	0.5	1.0 of 1: 10	0.3	0.1	0.5	2.5	PH

The second point to be determined is the anticomplementary effect of the antigen. In a good antigen this effect must not be evident in an amount considerably greater than the antigenic unit. A series of fifteen tubes is arranged as in Table 4, 0.1 c.c. of a known negative, inactivated serum is placed in each tube, one unit of previously titrated complement (usually 0.5 c.c.), increasing amounts of the antigen and enough salt solution to bring the total volume, when amboceptor and corpuscles are added, to 2.5 c.c. The tubes are now incubated for half an hour in the water-bath at 37 C., after which one unit of previously titrated amboceptor and 0.5 c.c. of corpuscles are added and the incubation continued for one hour. The tube which contains the largest amount of antigen in which hemolysis is complete is recorded.

The next and final point in the titration of antigen is the determination of the antigenic unit. This titration is identical with the determi-

nation of the anticomplementary value, except that instead of using a known negative serum, a known positive serum is employed. In fact, these two titrations may be, and usually are, carried out at one time. After the final incubation the tubes are placed in the ice-box for a few hours, when the tube is determined which contains the smallest amount of antigen in which inhibition of hemolysis is complete.

TABLE 5.—TITRATION OF ANTIGEN (ANTIGENIC UNIT)

Tube	Ser.	Comp.	Ant.	NaCl	Amb.	Corp.	Total	Results
1	0.1	0.5	0.5 of 1:100	0.8	0.1	0.5	2.5	H
2	0.1	0.5	0.6 of 1:100	0.7	0.1	0.5	2.5	PH
3	0.1	0.5	0.7 of 1:100	0.6	0.1	0.5	2.5	PH
4	0.1	0.5	0.8 of 1:100	0.5	0.1	0.5	2.5	PH
5	0.1	0.5	0.9 of 1:100	0.4	0.1	0.5	2.5	PH
6	0.1	0.5	0.1 of 1: 10	1.2	0.1	0.5	2.5	NH
7	0.1	0.5	0.2 of 1: 10	1.1	0.1	0.5	2.5	NH
8	0.1	0.5	0.3 of 1: 10	1.0	0.1	0.5	2.5	NH
9	0.1	0.5	0.4 of 1: 10	0.9	0.1	0.5	2.5	NH
10	0.1	0.5	0.5 of 1: 10	0.8	0.1	0.5	2.5	NH
11	0.1	0.5	0.6 of 1: 10	0.7	0.1	0.5	2.5	NH
12	0.1	0.5	0.7 of 1: 10	0.6	0.1	0.5	2.5	NH
13	0.1	0.5	0.8 of 1: 10	0.5	0.1	0.5	2.5	NH
14	0.1	0.5	0.9 of 1: 10	0.4	0.1	0.5	2.5	NH
15	0.1	0.5	1.0 of 1: 10	0.3	0.1	0.5	2.5	NH

The antigenic unit is that amount of antigen which is the average of the smallest amount which will completely inhibit hemolysis with a known positive serum, and the largest amount which will cause no inhibition with a known negative serum. For example, if we have found that 0.1 c.c. of the 1:10 dilution of antigen completely inhibits the hemolysis with a known positive serum, and 0.9 c.c. of antigen is the largest amount which does not inhibit hemolysis with a known negative serum, 0.5 c.c. is the antigenic unit.

ATTEMPTS TO PRODUCE SCARLATINA IN MONKEYS *

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Notwithstanding the numerous attempts to inoculate the lower monkeys with scarlet fever, the positive results reported, notably those of Cantacuzène[1] and Bernhardt,[2] have not been substantiated by others who have repeated the experiments of these observers. (Notes 1 to 4 give the complete recent literature.) Thus Landsteiner and Levaditi[3] inoculated thirty-five monkeys of five different lower species with exudate from the throat and with the blood and lymph-nodes of scarlet-fever patients, by local application to the fauces of the animals and by intravenous, intraperitoneal and subcutaneous injections of scarlet-fever products without obtaining a seemingly positive result. Klimenko,[4] making use of twenty monkeys of five lower species, was equally unsuccessful, although he included among his many experimental procedures that of Bernhardt, who claims to have produced the disease in fourteen out of nineteen aimals. Shortly after the publication of Cantacuzène's first papers, Dr. Park inoculated six monkeys with the blood of six fresh scarlet-fever cases, including several of the malignant type. The technic employed was the same as that described in Cantacuzène's report. The results[5] were completely negative. Our few experiments are given herewith as an additional contribution to the proof that this class of monkey is immune to the virus of scarlet fever when inoculated by any method heretofore made use of.

Some of the inoculations recorded had their inception in a laboratory accident: An assistant, while preparing the suspensions of streptococci for immunization of horses, sucked some of the suspension into her mouth. This accident occurred Oct. 24, 1912. On the morning of October 26, a beginning sore throat was noticed which became progressively worse during the day. The sore throat with general symptoms of prostration continued on October 28, tingling of the skin and arms and

* From the Research Laboratory, Department of Health, New York City.
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1. Cantacuzène: Compt. rend. Soc. de biol., 1911, lxxi, 196 and 405.
2. Bernhardt: Deutsch. med. Wchnschr., 1911, xxxvii, 791.
3. Landsteiner, Levaditi and Prasek: Ann. de l'Inst. Pasteur, 1911, xxv, 754.
4. Klimenko: Jahrb. f. Kinderheilk., 1913, lxxvii, 679.
5. Unpublished.

chest was noticed, probably the beginning of the exanthem. When seen on the morning of October 29, a general pharyngitis was present, and a typical scarlatinal rash covered the arms, chest and abdomen, the legs being comparatively free. The temperature was 101.6 F., rising to 102 later in the day. During the next two days the temperature fell to normal and the throat and general symptoms disappeared. The subsequent course was uneventful, desquamation taking place in a typical manner. As far as could be determined, there had been no contact with a case of scarlet fever.

The streptococci in the suspension were the following:

Scarlet fever—throat, 4 strains, 2 hemolyzing, 2 green.
Mastoiditis—3 strains, 3 hemolyzing.
Puerperal sepsis—3 strains, 3 hemolyzing.
Pleurisy—1 strain, green.
Erysipelas—1 strain, hemolyzing.

EXPERIMENTS

EXPERIMENT 1.—Monkey No. 593, Java, female, Nov. 25, 1912, throat swabbed and sprayed with a heavy suspension of strains of streptococci previously mentioned. This suspension was used in all the experiments. The blood-counts taken in all will be found in the accompanying table. No change in throat or tongue was observed in any animal, no inclusion bodies (Döhle), were seen in any of the smears except in Experiment 3.

Observations.—The temperature, which was taken for ten days before the animal was used to determine the normal range, fluctuated between 100 and 101.4 F. After inoculatioon the temperature range remained the same, except for one rise to 102.2, November 28.

EXPERIMENT 2.—Dog-faced baboon A, male. Dec. 3, 1912, throat swabbed and sprayed with the suspension.

Observations.—The temperature fluctuated between 99.4 and 101.6 F. for eight days preceding inoculation. After inoculation the highest point was 101.8.

EXPERIMENT 3.—Dog-faced baboon B, female. Dec. 13, 1912, throat swabbed and sprayed with suspension.

Observations.—The temperature before injection was only taken for three days and ranged about 101 F. After injection it rose at times to 102 and twice to 102.2 F. Small dot-like inclusion bodies were occasionally noted, which were slightly more numerous December 4.

Results.—Mixed streptococci from scarlet fever and other sources which have been grown for some months on media, caused no perceptible reaction, when applied to the throat of monkeys.

EXPERIMENT 4.—Monkey 593, Macacus, female. Dec. 6, 1912, 2 c.c. of sterile 10 per cent. sodium citrate solution were drawn into a syringe, which was then used to take 25 c.c. of blood from a child ill four and one-half days with scarlet fever. The child showed a profuse rash over body, typical scarlatinal tongue and throat and enlargement of the cervical, inguinal and axillary lymph-nodes. The temperature at the time was 102 F. The blood was injected intraperitoneally into the monkey within a few minutes after withdrawal from the child.

Observations.—Before and after injection the range of temperature fluctuated between 101 and 102.2 F. December 10, 11, 12 and 13, the temperature ranged from 102 to 102.2 F. At no other time did the temperature remain at this height.

The noteworthy points in this experiment are that on the fourth day after the injection the temperature reached its highest point and remained high for four days. On the day preceding this rise the polynuclear percentage rose and during the four days of highest temperature continued its rise with that of the total count. As the monkey was menstruating at this time no weight can be placed on these changes. At best they were too slight to be even suggestive of a successful inoculation.

TABLE OF TOTAL NUMBER OF LEUKOCYTES AND PERCENTAGE OF POLYNUCLEARS IN EXPERIMENTS 1 TO 7

	Animal									
	593		A		B		593*		A*	
Days Before Inoculation	Leuk. No.	Polyn. %	Leuk. No.	Polyn. %	Leuk. No.	Polyn. %	Leuk. No.	Polyn. %	Leuk. No.	Polyn. %
8	—	—	12,600	43	—	—	—	—	—	—
7	—	—	15,600	27	—	—	—	—	11,500	38
6	13,850	59	13,150	40	—	—	—	—	—	—
5	10,500	67	—	—	—	—	—	—	—	—
4	12,000	63	11,000	33	—	—	10,500	57	10,500	44
3	12,500	64	—	—	—	—	—	—	—	—
2	13,000	65	—	—	11,000	48	10,700	53	—	—
1	10,500	64	14,000	40	14,000	35	—	—	—	—
Day of Inoculation										
0	13,300	68	—	—	—	—	—	—	—	—
Days After Inoculation										
1	13,600	59	12,400	36	12,500	54	11,300	56	13,000	43
2	11,300	66	12,000	42	—	—	—	—	—	—
3	—	—	—	—	13,000	46	12,500	70	9,000	54
4	11,200	62	11,500	38	11,000	50	16,500	68	9,000	37
5	—	—	—	—	13,000	33	14,000	70	10,400	31
6	—	—	—	—	—	—	10,500	61	—	—
7	10,500	57	10,500	44	—	—	12,500	68	—	—
8	—	—	—	—	10,200	—	9,000	59	9,100	—
9	10,700	53	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	9,300	52	—	—
12	—	—	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	10,300	—	—	—

* Second inoculation.

EXPERIMENT 5.—Dog-faced baboon A. Dec. 13, 1912, 25 c.c. of blood were taken from a child of 13, five days ill with scarlatina, who showed a typical rash and scarlatinal throat and tongue. The temperature at the time was 100 F. The blood was injected intraperitoneally into the monkey immediatel after withdrawal from the child.

Observations.—The temperature reached 101.8 before injection, but as a rule fluctuated closely around 101 F. After injection the range was between 101 and 102 F., giving in the chart the appearance of a slight transient rise.

Result.—The intraperitoneal injection of 25 c.c. of blood into a macacus monkey was followed by a slight rise in temperature and a coincident increase in the total and polynuclear leukocyte count; this rise, however, was slight and probably due to other causes. A similar injection into a baboon was followed by no reaction.

EXPERIMENT 6.—Dog-faced baboon 12, female. March 31, 1913, swabs were smeared with the discharge from ears, throat and nose of seven children, ill from four to fifty-five days with scarlet fever alone or with scarlet fever and subsequent complications. The material on the swabs was washed off in sterile normal saline solution. This suspension was filtered through a Berkefeld filter, the first filtrate being thrown back on the filter and saline passed through the filter after the suspension had passed through. Forty c.c. of filtrate was obtained, which was bacterially sterile, and this was injected intraperitoneally.

Observations.—The temperature rose to 102 F. the second day after injection. The normal range for this animal was about 101 F., occasionally rising half a degree higher.

EXPERIMENT 7.—Dog-faced baboon 12, female. April 21, 1913, swabs were collected as in the preceding experiment. These were washed off in saline, giving a total of 4 c.c. suspension. This was sprayed and swabbed on the animal's throat.

Observations.—The temperature did not rise above 101.6 F.

Results.—The intraperitoneal injection of bacteria-free material from scarlet-fever cases and the direct application to throat of the same type of material without filtration, were followed by no reaction.

SUMMARY

The attempt to produce scarlet fever in monkeys and baboons by the faucial inoculation of streptococcus cultures or unfiltered exudates and discharges from scarlet-fever cases, as well as the intraperitoneal injections of filtrates of such exudates and discharges and of large quantities of blood, gave negative results.

GERMAN MEASLES (RUBELLA): AN EXPERIMENTAL STUDY *

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Investigations as to the nature of German measles are totally lacking. This is probably due to the fact that this disease is mild and so rarely encountered in hospital wards. The only opportunity afforded for a systematic study from a laboratory standpoint is the occurrence of an epidemic in an institution where children are housed in large numbers. An occasion of this kind—an epidemic attacking fifty or more children in an infant asylum—led to the present investigation, which includes a bacteriological study of the blood, inoculations of blood into monkeys with the object of producing the disease, and a cellular examination of the blood during the period of incubation of the disease.

In four instances the blood was obtained for bacteriological examination. In three of the cases the rash had been present for less than twenty-four hours, and in the fourth for about thirty-six hours; in two the temperature of the child was above the average, that is to say, between 102 and 103 F. So that it may be said that I was able to obtain the blood in severe cases as well as at the very onset of the disease. The latter circumstance would appear of particular importance when we remember that the virus of measles has been found with regularity only in the early period following the stage of exanthem. Numerous cultures made on mediums containing serum and hemoglobin showed no growth whatsoever. Other cultures were made by Dr. Zingher, whom I wish to thank in this connection, according to the method developed by Noguchi for the cultivation of the spirochete of syphilis; the medium contains two parts of salt-free agar, one part of ascitic fluid, fresh kidney tissue, and is covered with liquid petrolatum to insure anaerobic conditions. It was not found possible to obtain any growth from the inoculation of the blood in our cases. The tubes were incubated for some weeks.

An attempt was made to produce this disease in monkeys. Four *Macacus rhesus* monkeys were inoculated with blood obtained from four different cases. In two instances the blood was from the same source as that used for purposes of cultivation. In all cases the rash had existed less than twenty-four hours; in most the temperature was high when the blood was withdrawn. Following is a summary of the protocols:

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* From the Research Laboratory, Department of Health, New York City.
* Submitted for publication Jan. 8, 1914.

CASE 1.—Feb. 7, 1913. Child which developed rash during the night; temperature normal.

Three c.c. of citrated blood inoculated intraperitoneally into Monkey 1. The temperature was followed for four weeks. Although it frequently rose to 103 or over, there was no sudden rise or fall.

CASE 2.—March 3, 1913. Rash seen on child for first time this morning; temperature 101 F.

Five c.c. of blood obtained from right jugular vein, and inoculated intraperitoneally a few hours later into Monkey 2. The course of the temperature is shown in the accompanying chart. It will be noticed that there is a definite rise occurring on the nineteenth day after inoculation and lasting for about forty-eight hours.

CASE 3.—March 6, 1913. Child with rash for past twelve hours; temperature 101 F.

Four c.c. inoculated intraperitoneally into Monkey 3. For three weeks temperature varied between 101.1 and 102.3 F. No rash.

CASE 4.—March 8, 1913. Temperature of patient 101.3 F. when blood was withdrawn. Rash had existed about twelve hours.

Eight c.c. of blood inoculated intraperitoneally into Monkey 4; cultures also taken. For the next three weeks the temperature ranged between 101.3 and 103 F. No exanthem.

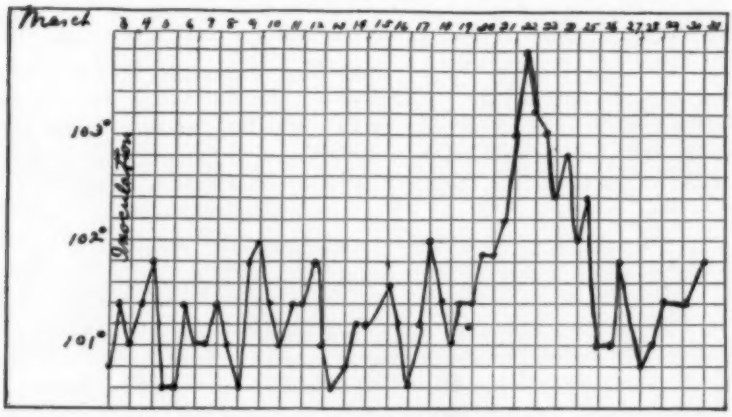

Chart of temperature of monkey (Case 2) inoculated with blood from early case of rubella.

It will be seen that in each instance from 3 to 8 c.c. of blood was inoculated intraperitoneally. In no case did the rash develop. In view of the fact, however, that this appears so rarely and faintly in experimental measles, it was hardly anticipated that we would succeed in producing a rash experimentally in rubella. Our attention, therefore, was closely directed to the course of the temperature. In only one instance was there a rise in temperature, following a considerable period of incubation (see chart). In the others there was no such rise. In view of the fact that the temperature of monkeys is somewhat unstable, too great significance should not be accorded this rise of temperature. In general,

it may be stated that there is danger in placing too great reliance on a rise of temperature as the sole criterion of the production of a disease in monkeys. If the rise is sudden and the period of incubation carefully controlled, however, this method of study is of value.

Although there have been no cultures whatsoever made of the blood in German measles and no inoculation experiments on monkeys, there have been, as might be supposed, some previous investigations as to the number and type of leukocytes in this disease. Hildebrandt and Thomas[1] found a leukopenia at the onset of the exanthem, and an increase of the lymphocytes, as well as an increase in the large Türk cells. Michaels,[2] as the result of an investigation of eighty cases of rubella, also found a leukopenia in the majority of cases. These studies, however, were carried out after the rash had appeared; and the only study which has taken cognizance of the blood in the earlier stages of the disease was one by Plantenga.[3] Our investigation concerned itself entirely with the blood during the incubation period. This was very easily carried out in the institution; for as soon as rubella developed, blood-smears were taken daily from the healthy children in the ward and were laid aside for examination in the event of some of these children developing the disease. In this way, in numerous instances, consecutive blood examinations were carried out before and up to the appearance of the rash. The following data show the results of these examinations, which were kindly carried out for me by Dr. Jerome Zuckerman:

1. H. B., 2 years old. Exanthem May 2.

Per Cent. of	April 28	April 29	April 30	May 2
Polyn.	55	66	53	50
Lymph.	20	22	35	35
L. mono.	11	5	8	10
Eosinoph.	3	4	4	4
Basoph.	1	3	0	1

2. L. S., 3½ years old. Exanthem May 2.

Per Cent. of	April 28	April 29	April 30	May 2
Polyn.	59	51	54	41
Lymph.	28	33	36	45
L. Mono.	5	9	9	9
Eosinoph.	8	7	1	4
Basoph.	..	..	..	1

3. S. S., 5 years old. Exanthem May 2.

Per Cent. of	April 28	April 29	April 30	May 2	May 12
Polyn.	49	46	50	31	50
Lymph.	35	39	40	55	34
L. Mono. ...	7	5	4	5	8
Eosinoph. ..	5	10	5	6	4
Basoph.	4	0	1	3	4

1. Hildebrandt and Thomas: Ztschr. f. klin. Med., 1906, lix, 444.
2. Michaels, M.: Arch. Pediat., 1908, xxv, 598.
3. Plantenga: Arch. de méd. d. enf., 1903, vi, No. 3.

4. M. G., 2 years old. Exanthem June 14.

Per Cent. of	June 11	June 13	June 14	June 19
Polyn.	43	33	34	53
Lymph.	46	57	56	42
L. Mono.	7	7	7	5
Eosinoph.	4	3	3	

5. G. S., 2 years od. Exanthem June 19.

Per Cent. of	June 14	June 18	June 19
Polyn.	40	34	29
Lymph.	51	62	63
L. Mono.	8	1	7
Eosinoph.	..	3	1

6. R. K., 2½ years old. Exanthem June 19.

Per Cent. of	June 14	June 18	June 19
Polyn.	52	35	45
Lymph.	45	59	51
L. Mono.	3	6	4

7. W. S., 2 years old. Exanthem June 19.

Per Cent. of	June 14	June 18	June 19
Polyn.	54	46	45
Lymph.	41	52	54
Eosinoph.	5	2	1

It is evident that *in almost all cases of German measles there was a definite increase in the lymphocytes even preceding the appearance of the exanthem.*

It would seem that this premonitory lymphocytosis might be of value to institutions in heralding the fact that infection has taken place, and in facilitating the segregation of infected from non-infected children, and thus, perhaps, in limiting the spread of an epidemic. That there is a lymphocytosis at the time of the appearance of the rash may also serve to differentiate rubella from scarlet fever, a disease with which it is sometime confounded, but which is associated with an increase of polynuclear cells in its early stage.

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THE DIFFERENTIAL DIAGNOSIS OF THE INTESTINAL ENTAMEBAS OF MAN*

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The observations on the parasitic amebas that have been made within recent years have emphasized the importance of distinguishing between the species parasitic in the intestine of man, and it is the purpose of this contribution to give, as briefly as possible, the chief morphological points on which such a differentiation must rest, and the technic that I have found most helpful in demonstrating the morphology of these parasites. In order that what follows may be clear, a brief review of our present knowledge of the intestinal entamebas of man is necessary.

HISTORICAL ACCOUNT

In 1903, Schaudinn[1] described and differentiated two species of entamebas parasitic in the intestine of man. One of these, the cause of the disease known as amebic dysentery, he named *Entamoeba histolytica*, while the other, a harmless commensal, he named *Entamoeba coli*.

In 1905, while studying these parasites in soldiers invalided home from the Philippine Islands with dysentery, I confirmed Schaudinn's observations, and published my results in the summer of that year.[2] In that paper I detailed the morphology of both species and the results of experiments on kittens with these entamebas, being the first to confirm Schaudinn's statements regarding the pathogenicity of *E. histolytica*, and the non-pathogenicity of *E. coli* to these animals. At that time I suggested that the ameba concerned in the causation of dysentery be named *Entamoeba dysenteriae*, but as the name *dysenteriae* was afterward shown by Stiles[3] to be merely a synonym of *coli*, I adopted Schaudinn's name *histolytica* for this parasite.

Hartmann,[4] in 1906, confirmed Schaudinn's classification of the intestinal entamebas of man, but for several years the distinction between *E. coli* and *E. histolytica* was disputed by most authorities, largely owing

* From the U. S. Army Central Department Laboratory.
* Submitted for publication Jan. 8, 1914.

1. Schaudinn, F.: Arb. a. d. k. Gsndhtsamte, 1903, xix, 547.
2. Craig, Charles F.: Am. Med., 1905, ix, 854, 897, 937.
3. Stiles, C. W.: Proc. Am. Public Health Assn., 1905, xxx, 292.
4. Hartmann, M.: Arch. f. Protistenk., 1909, xviii, 207.

to the observations of Musgrave and Clegg[5] on the amebas they secured on artificial culture-mediums and their conclusion that these amebas were identical with those found parasitic in the intestine of man, and "that all amebas are or may become pathogenic."

In 1907, Viereck[6] described a species of ameba observed in certain cases of dysentery which differed markedly from the original description of *E. histolytica* and which he regarded as a new species, naming it *Entamoeba tetragena*. His observations were confirmed by Hartmann,[7] Werner,[8] Darling,[9] Whitmore,[10] and myself, but further study has shown that in reality *E. tetragena* is identical with *E. histolytica*, a portion of the life cycle of the latter parasite having been erroneously described by Schaudinn, Hartmann and myself, as well as by others.

Walker[11] was actually the first to point out that *E. tetragena* is identical with *E. histolytica*, and all of the work that has been accomplished by other investigators since his paper appeared has simply been confirmatory of his conclusions, and at present all students of the subject, who have had sufficient material on which to work, are agreed that two well-defined species of entamebas occur in the intestine of man, *E. histolytica* and *E. coli*. The first is the cause of amebic dysentery, while the second is a harmless commensal, occurring in the intestines of a varying percentage of individuals, in both health and disease, in almost every part of the world.

In 1905 and later, I stated[12] that the parasitic amebas of man had not been cultivated, nor could they be cultivated, on the ordinary culture-mediums employed by those who claimed to have cultivated these organisms from the feces or other material, and this statement has been amply confirmed by nearly every subsequent investigator. At present there is no adequate proof that a single species of entameba has ever been cultivated on any artificial medium. A few observers still claim that the amebas that they have succeeded in cultivating are identical with the entamebas of the human intestine but their claims have been refuted by many investigators and it has been conclusively shown that the amebas so cultivated are entirely distinct from the entamebas of man, and have nothing to do with the production of dysentery.

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5. Musgrave, W. E., and Clegg, M. T.: Rep. Bur. Govt. Lab., Biol. Lab., Manila, 1904, No. 18.
6. Viereck, H.: Arch. f. Schiffs u. Tropen-Hyg., 1907, xi, 1.
7. Hartmann, M.: Arch. f. Protistenk., 1912, xxiv, 163.
8. Werner, H.: Indian Med. Gaz., 1909, xliv, 241.
9. Darling, S. T.: Jour. Trop. Med., 1912, xv, 1.
10. Whitmore, E.: Arch. f. Protistenk., 1911, xxiii, 7.
11. Walker, E. L.: Philippine Jour. Sc., Sec. B, 1911, vi, 259.
12. Craig, Charles F.: Am. Med., 1905, ix, 854, 897, 937; Jour. Infect. Dis., 1908, v, 324; The Parasitic Amebae of Man, Philadelphia, 1911, p. 58, Jour. Infect. Dis., 1913, xiii, 1.

Since 1905 I have consistently maintained the specificity of *E. histolytica* and *E. coli*, and in my first paper, and since, have insisted that it is possible to differentiate these two species in material containing them. I have also proved that neither of these parasites can be cultivated on ordinary culture-mediums, and that, while *E. histolytica*, when fed to kittens or injected into the rectum, produces typical amebic dysentery and even liver abscesses, *E. coli* is harmless to them. Many observers have confirmed my conclusions; but the recent publication of Walker and Sellards'[13] experimental work on man with both cultural and parasitic amebas definitely proves every point for which I have contended for years. They have shown conclusively that the amebas that can be cultivated from water, from the feces of man and other animals and from other sources, are incapable, when ingested, of producing the symptoms or lesions of dysentery in man, and that they are not entamebas but free-living amebas, having nothing to do with the production of dysentery and unable to live as parasites in man. They have also proved beyond dispute that *E. histolytica* and *E. coli* are distinct species, and that when fed to man, *E. histolytica* produces the symptoms and lesions of amebic dysentery, while *E. coli*, though a parasitic species, is harmless.

While all of the essential points covered in this paper have been previously proved by experimentation on the lower animals, the results obtained by Walker and Sellards possess the very great advantage of having been worked out on man, the natural host of *histolytica* and *coli*, and, in my opinion, settle for all time the question of the relation of parasitic and cultural amebas to disease in man.

THE IMPORTANCE OF DIFFERENTIATING E. HISTOLYTICA AND E. COLI

In discussing the importance of differentiating between *E. histolytica* and *E. coli*, Walker[14] states that such differentiation is possible from a study of the morphology and life-cycle of these parasites, an opinion which I have constantly advocated, and which has been steadily gaining ground among students of the subject since the paper of Schaudinn first appeared, although some investigators, in their writings, have given the impression that such a differential diagnosis is a matter of the very greatest difficulty, only to be attempted by a protozoologist. While I would be the last to state that these parasites can be differentiated by one with little or no experience in the study of intestinal protozoa, I do believe that the study of a few suitable cases of infection should enable one to differentiate the common intestinal entamebas of man. Recent researches have shown that there are but two species of importance

13. Walker, E. L., and Sellards, A. W.: Philippine Jour. Sc., Sec. B, 1913, viii, 253.

14. Walker, E. L.: Philippine Jour. Sc., Sec. B, 1911, vi, 259.

occurring in man and I am convinced that any one trained in the use of the microscope and in the study of the common bacteria and protozoa of man should have no difficulty in recognizing *E. histolytica* or *E. coli*.

The importance of differentiating these parasites can hardly be overestimated, and attention was called to it in my first paper published in 1905. Our statistics regarding the occurrence of amebic dysentery are very largely valueless because but little effort has been made to identify the species of entameba present, numberless infections with *E. coli* being mistaken for infections with *E. histolytica* and diagnosed as amebic dysentery, whether symptoms were present or not. In fact, some practitioners have gone so far as actually to treat for amebic dysentery every person showing an ameba in the feces, even in the absence of any effort to identify the ameba present, and very wonderful records of successful treatment of this disease have been thus built up on mistaken diagnoses. The mere finding of an ameba in the feces means nothing unless the species be identified, and at present a diagnosis of entamebic dysentery based on anything else than the identification of the entameba present as *E. histolytica* is not only unscientific but unjustifiable.

It is now well known that *E. histolytica* may live in the intestine of man for weeks, or even months, before symptoms of its presence are noticed. Persons thus harboring the parasite are efficient "carriers" of the infection, and undoubtedly are largely responsible for the spread of the disease. The great importance of the recognition of such infections is evident, and to recognize them it is necessary to be able to differentiate this entameba from the harmless *E. coli*, so commonly present in most tropical and subtropical regions in healthy persons. The importance of the discovery of these "carriers" emphasizes the great value of a routine examination of the feces of people living in regions in which entamebic dysentery is endemic, even when no symptoms of intestinal disease are present, and may be compared with the importance of the examination of the blood of both healthy and diseased persons in malarial regions in the prophylaxis of that disease. Not only will the carriers of the infection thus be recognized, but treatment can be instituted when it will prove most efficient, that is, before severe lesions have been produced in the intestine.

The differential diagnosis of *E. histolytica* and *E. coli* depends on a careful study of the morphology and life-cycle of these parasites both in the living condition and in properly stained preparations. While I believe that one trained in the study of these organisms can differentiate them in the living condition, it is always best to confirm such a diagnosis by the study of a stained preparation. The methods given in the following section are those I have found most useful in my own work and all of them can be followed by anyone trained in laboratory technic. I have

not endeavored to give a large number of methods of staining, as I have found that it is much better to perfect oneself in one or two methods rather than to try to master all that may have been recommended.

TECHNIC

The intestinal entamebas of man may be studied either in the living condition or in stained preparations, and with either method of examination a differential diagnosis between *E. histolytica* and *E. coli* can be made. The study of the living parasites has been sadly neglected by some protozoologists, largely because of lack of material, and I am firmly of the opinion that this neglect has led to many mistakes in the interpretation of vital phenomena because of the great reliance these authorities have placed on the morphology of the stained entamebas. Indeed, some investigators have gone so far as to state that these parasites should only be studied in stained preparations, a statement which appears absurd to one who has had the opportunity of studying many living preparations, for it is just as easy to differentiate these parasites in the living condition as when stained, if one has had sufficient experience.

While, as I have already stated, it is always well to confirm one's species diagnosis by stained specimens, I am satisfied that in most cases a diagnosis of *E. histolytica* or *E. coli* can be made without recourse to staining the organisms and that this is the best and simplest method of diagnosing all types of intestinal infection with entamebas.

Preparation of Living Specimens.—In examining feces for living entamebas a small portion of a freshly passed stool is picked up with a platinum loop, placed on a microscopic slide, covered with a cover-glass, and gentle pressure exercised until the material is evenly spread. Care should be taken not to make too thick a preparation and several should be examined before a negative result is reported. I have repeatedly found a few entamebas in the ninth or tenth preparation examined, and sometimes I have had to examine as many as fifteen preparations before entamebas were discovered.

The feces may be examined in the normal condition, that is, without previous purgation, or a purgative may be administered before the examination. In my own experience I have obtained the best results after the administration of a purgative, especially in the demonstration of infections with *E. coli*, but Walker[15] advocates the examination of stools without previous purgation, because of the greater ease with which a differential diagnosis between *E. histolytica* and *E. coli* may be made. This has not been my experience and I would recommend the administration of a saline cathartic before the examination in all cases in which the stools are well formed. If partially formed or formed stools are to be examined it is necessary to dilute a small portion of the feces with normal salt solution or the preparation will be too opaque for examination.

It is always best to examine the feces immediately after passage for in such specimens the vegetative stages of the parasites, if present, are motile and therefore easily recognized. Many authorities have stated that the diagnosis of an entamebic infection should never be made unless the entamebas are observed in a motile condition; but such advice is pernicious, in that it would result in over-

15. Walker, E. L.: (With Sellards) Philippine Jour. Sc., Sec. B, 1913, viii, 253.

looking all latent and chronic infections in which the entamebas are encysted and, of course, motionless. As the recognition of the latent cases or "carriers" is of the greatest practical importance from a prophylactic point of view, it can be easily understood that such a statement might result in great harm. As a matter of fact, to one who has had sufficient experience in the study of these parasites, it is just as easy to recognize a non-motile entameba as a motile one, provided degenerative changes have not occurred, and, as the cysts of entamebas persist unchanged for several hours in the feces, one may demonstrate them in material that has been collected for some time. In fact, in the tropics and subtropics, I have often observed motile entamebas in feces that had been kept at room temperature for several hours.

If the feces are to be transported for any distance before the examination is made, they should be placed in a warm vacuum bottle or similar receptacle. No disinfectant should be mixed with stools that are to be examined for entamebas.

After preparing the specimen for examination as directed, the cover-glass should be ringed with petrolatum, and the specimen examined first with a $\frac{1}{6}$-inch objective and a 1-inch eye-piece, and later with the $\frac{1}{12}$-inch oil-immersion objective. If one is experienced in the examination of feces for entamebas much time will be saved by going over the specimen with the $\frac{2}{3}$-inch objective and 1-inch eye-piece, picking out the suspicious objects, and then examining them with the higher power objectives; but considerable experience is required to recognize the entamebas with the lower-power lens mentioned.

If the preparation contains motile entamebas, they are generally easily recognized even by one having little or no experience in the study of these organisms, but the non-motile, precystic and encysted forms, while very distinctive to an experienced observer, may be easily confused by the inexperienced with other bodies occurring in the feces. Attention to a few simple points, however, will serve to distinguish entamebas from other objects found in the feces. In the resting stage these parasites are larger than pus-cells or protozoa that could be confused with them, are more refractive in appearance, and are characterized by a nucleus which is small in size when compared with the nucleus of other cells, and which consists of a very refractive ring of minute granules enclosing a homogeneous substance. If blood is present in the feces the red blood-corpuscles are often observed within *E. histolytica* and this serves at once to distinguish this parasite from other cells or protozoa.

When the entamebas are encysted they are larger than the cysts of other intestinal protozoa, are spherical or oval in shape, very refractive, and possess a distinct cyst-wall. From two to eight or more nuclei are present within the cyst. It is comparatively easy to distinguish the cysts of *E. histolytica* from those of *E. coli*, as will be explained later.

The use of a warm stage or an incubator for holding the microscope is not necessary unless one desires to study the life-cycle of entamebas while living, when some apparatus that will keep the organisms at body temperature is absolutely necessary. Much may be learned regarding their vital activities from the study of living entamebas in this manner.

Preparation of Stained Specimens.—In structure the entamebas are very delicate organisms and it is undoubtedly true that any method of fixing and staining them will cause some distortion and morphological change, so that it is essential, if one desires the best results, to select staining methods that have been found to produce the least artificial change in the morphology of the parasites.

In 1905 I recommended[16] the use of Wright's stain, after dry fixation, as a useful clinical method of differentiating *E. histolytica* from *E. coli*, and my observations regarding the use of this modification of the Romanowsky stain have since been confirmed by James,[17] who has secured excellent results by double stain-

16. Craig, Charles E.: Am. Med., 1905, ix, 854, 897, 937.
17. James, W. M.: New York Med. Jour., 1913, xcvii, 702.

ing with Hastings' and Giemsa's stains. While these stains, however, preceded by dry fixation, will suffice for the differential diagnosis of these parasites, much better results, as regards morphological detail, are secured by wet fixation, followed by one of the hematoxylin stains, and as some of these methods are but little more difficult of application than the modifications of the Romanowsky stain, I believe that they should be generally adopted when stained preparations of the entamebas are to be studied.

To prevent alteration in morphology at any stage of fixation or staining rapid handling and the use of the process of wet fixation are necessary, as drying markedly distorts the nuclear structure of the entamebas on which a considerable part of our differential diagnosis depends.

I believe that it is much better to make the smears of the material to be examined on microscopic slides, although some authorities prefer cover-glasses. The slides are easier to handle and a much larger smear may be made, thus increasing the chances of finding well-stained entamebas. Some form of jar should be used in which to place the various solutions used in the process of fixing and staining, to which the preparations are transferred during staining, and for this purpose I have found the Coplin staining-jar, which may be obtained from any dealer in microscopical goods, an excellent one.

The same directions should be followed in selecting material from the feces to be stained for entamebas as have been already described for making living preparations; but the smear must be made very rapidly and placed immediately in the fixing solution, to prevent drying, which, as remarked, will render it useless. The material may be smeared in various ways, but I have found that the simplest method is to place a small portion of the material to be examined near one end of the slide, and then to draw it forward over the slide with the end of another slide applied nearly at right angles to the slide containing the material. A little practice will be found necessary before thin, even smears are obtained, but the method is much better than rubbing the material over the slide with a platinum loop.

The character of the material examined has much to do with the success of smearing with a slide, for if much mucus be present it will be found impossible to spread it evenly in this manner, and then it will be necessary to use the platinum loop. If the feces are of solid consistence, an emulsion of a portion should be made with normal salt solution, and this emulsion used for making the smears. James recommends making the smears with a camel's-hair brush and this is an excellent method if little mucus is present. When the entamebas are few in the material to be examined, as determined by a preliminary microscopic examination, a portion may be diluted with warm normal salt solution, centrifuged, and smears then made from the sediment.

The most important points to bear in mind in making the smears are that they should be as thin as possible and that they should be placed in the fixing solution immediately.

Fixing the Preparations.—Many solutions have been recommended for fixing entamebas, but I have found that Schaudinn's sublimate-alcohol mixture answers every requirement and is a suitable mixture for use with any of the hematoxylin stains.

This mixture consists of two parts of a saturated solution of mercuric chlorid in normal salt solution, and one part of absolute alcohol. The mercuric chlorid solution is made by adding the mercury to boiling normal salt solution until saturation occurs. This may be kept indefinitely as a stock solution.

The mixture with the alcohol is made just before use and should be kept at the body temperature during the fixing process. The addition of 3 per cent. of glacial acetic acid improves the fixing qualities of the solution, but is not necessary.

Immediately after the smears are made they are immersed in this fixing fluid, which is heated to 37 C (98.6 F.), and fixed for five minutes. They are then

transferred to jars containing various strengths of alcohol in the manner indicated in the following scheme:

In sublimate-alcohol, at 37 C. for five minutes; 50 per cent. alcohol for five minutes; again in 50 per cent. alcohol for five minutes; 70 per cent. alcohol for fifteen minutes; iodin alcohol (alcohol to which enough tincture of iodin has been added to produce a brown color) thirty minutes; 70 per cent. alcohol, until the yellow color given by the iodin disappears; 95 per cent. alcohol, thirty minutes; absolute alcohol, fifteen minutes; 80 per cent. alcohol, five minutes; 50 per cent. alcohol, ten minutes, and finally in distilled water, where they may be kept until stained.

The entire process should be carried out in staining-jars and only a few preparations handled at a time.

While the preceding process may seem complicated, it will not be found so in practice and the time consumed will be amply repaid by the excellent results obtained. The preparations must be carried rapidly from one solution to another as at no time must drying occur.

Staining the Preparations.—After the smears have remained in distilled water for ten minutes or longer they are ready for staining and the method selected will depend largely on the experience of the observer. I have found that the ferric chlorid hematoxylin stain of Mallory gives excellent results, and this is the method I would recommend for routine work. This stain and the method of applying it were first brought to my attention in the study of the entamebas by Dr. W. M. James of Ancon, and I have found both most valuable in the differentiation of *E. histolytica* and *E. coli*. In the following (Dr. James') method of staining, as in fixing, the greatest care must be taken that the smears do not dry at any stage of the staining process:

1. Having washed the preparations thoroughly in distilled water, place them on glass rods arranged over a large dish or jar, and pour over them a 10 per cent. aqueous solution of ferric chlorid, so that the preparation is flooded. This is the mordant and should be allowed to remain on the preparations for from ten to fifteen minutes.

2. Drain the preparations, one by one, and pour over them at once a 1 per cent. aqueous solution of hematoxylin, prepared by dissolving hematoxylin crystals in boiling distilled water until the mixture is cherry-red. The hematoxylin solution should be freshly prepared and just warm to the hand when used. As soon as the hematoxylin is added the mixture becomes black and the smear disappears from view; but enough hematoxylin should be added to render the smear just visible through the brownish-yellow solution covering it. The preparations are now allowed to stain for from five to ten minutes.

3. Wash the preparations thoroughly in distilled water by pouring a considerable amount over the smears or by placing them in at least two changes of distilled water contained in a staining-jar.

4. Decolorize in a 0.5 per cent. ferric chlorid solution, the decoloration being controlled by microscopic examination. When the majority of the entamebas show a well-defined nucleus having a distinct membrane and contaning chromatin granules, decoloration has reached the proper point and should be stopped. This is accomplished by washing in distilled water. If the preparations are very thick it will be necessary to use a 1 per cent. solution of ferric chlorid in decolorizing.

5. After decolorization, the preparations are rinsed in distilled water, washed in running tap-water for fifteen minutes, dehydrated in 95 per cent. and absolute alcohol, cleared in xylol, and mounted in acid-free xylol balsam. If desired, the preparations may be left in water for several hours, and they should remain in each of the alcohols for at least fifteen minutes.

While the preceding stain gives excellent results and is all that is necessary in routine work, more nearly perfect results, as regards the differentiation of nuclear structure, may be secured with the iron-hematoxylin method of Heidenhain. This method of enabling the morphology of protozoa to be studied has never been surpassed; but it requires considerable technical ability and experience before good results are obtained.

Two solutions are used in this method which is, in brief, the following:
Solution 1:

A 2.5 per cent. solution of iron alum. (Ferric ammonium sulphate.)
Solution 2:

Hematoxylin crystals	1 gm.
Absolute alcohol	10 gm.
Distilled water	90 gm.

The hematoxylin is dissolved by heat in the water and the alcohol added when the mixture is cold, after which it is placed in a dark glass bottle lightly corked with cotton, and allowed to ripen for a month or six weeks, at the end of which time it is tightly corked and ready for use.

Method of Using.—After fixing, as described, in sublimate-alcohol solution, the preparations are thoroughly washed in distilled water, and placed in Solution 1 for from eight to ten hours or over night; they are then rinsed in distilled water and placed in Solution 2, where they should remain for from twenty-four to forty-eight hours, after which they are rinsed, one by one, in distilled water, and placed again in Solution 1. When this is done dense black clouds come from the preparation but in from three to four minutes these almost disappear and only slightly blackish clouds are formed. When this point is reached the preparations should be washed in distilled water, and placed in a jar containing tap-water. The preparation should now be examined by mounting it in water with a cover-glass and using a high-power dry lens (a 1/6 or 1/8) and a 1-inch eyepiece. If the entamebas show a clearly defined nucleus containing chromatin granules, the decolorization has been sufficient, but if not, the preparation is again washed in distilled water, placed in Solution 1 for a second or two, then mounted with a cover-glass in Solution 1 and examined under the microscope. As soon as the entamebas show a well-defined nucleus, the specimen should be rinsed in distilled water, dehydrated for ten minutes each in 50, 70 and 80 per cent. alcohol, in 95 per cent. alcohol for fifteen minutes, and in absolute alcohol for twenty minutes; cleared in xylol and mounted in acid-free xylol balsam.

While the Heidenhain stain is most reliable in experienced hands, I am of the opinion that more mistakes have been made in the interpretation of the morphology of entamebas stained in this manner than with any other stain that has been used for this purpose. It is very easy to carry the process of decolorization too far, and to make the nucleus of *E. coli* resemble that of *E. histolytica* and I have repeatedly observed mistakes made by the best protozoologists in diagnosing between these species in preparations stained by the iron-hematoxylin method. For this reason the Heidenhain method is not so generally useful as the Mallory stain, for while some experience is required, even in the use of the latter stain, it is not so difficult to manipulate and, to secure good results, requires much less experience in its use.

It should be remembered, however, that no staining method is as useful in diagnosis as the examination of the living parasites, and while such methods are absolutely essential for the study of the minute morphology and reproductive cycle of the entamebas they are not essential either in the differentiation of *E. coli* and *E. histolytica* or in the clinical diagnosis of entamebic infection.

When entamebas are well stained, by either of the methods here mentioned, the cytoplasm is of a grayish color, while the nucleus is well defined, the nuclear membrane and chromatinic granules being stained a jet black. The entamebas may be confused, when stained, with other protozoa occurring in the feces or with animal or vegetable cells. It is seldom difficult for one who has had experience in the study of these parasites to differentiate them from other organisms or cells, and the cysts are very characteristic, by reason of their multiple nuclei. There are some vegetable cells having two or more nuclei that are often confusing to the inexperienced, being mistaken for the cysts of entamebas, but reference to the descriptions of the morphology of the true entamebic cysts should prevent such confusion. Certain protozoa, as *Trichomonas hominis, Lamblia intestinalis* and *Balantidium coli* might be confused with the intestinal entamebas at certain stages in their development, but such a mistake could be made only by one having a very superficial knowledge of protozoology. The *Blastocystis hominis,* a vegetable cell of uncertain origin, has frequently been mistaken for encysted entamebas, but differs greatly in morphology, only a small portion of the cell being occupied by the cytoplasm, which contains a few granules of material giving the staining reaction of chromatin.

A considerable number of pages might be written describing in detail the bodies of various kinds that may be confused with the intestinal entamebas, but only experience in this line of work will prevent mistakes, and this is better gained by the study of actual preparations than by reading about differential details.

THE DIFFERENTIAL DIAGNOSIS OF E. HISTOLYTICA AND E. COLI

The most recent observations on the intestinal entamebas have apparently proved that they pass through at least three distinct stages in their life-cycle when morphological changes occur of such importance that, unless one is well aware of them, grave errors may follow in their interpretation, and the species thus be confused. This has been proved by the number of years that elapsed before it was recognized that the so-called *E. tetragena* is actually but one stage in the development of *E. histolytica,* this being entirely due to false interpretation of the morphology and life-cycle of the parasite. The slavish insistence by some

protozoologists on the absolute value of nuclear structure in the differentiation of the entamebas has been largely responsible for the mistakes that have been made in the study of these parasites, and it is questionable whether our knowledge has been advanced or delayed by the studies of the morphologists who have devoted their energies to the study of stained entamebas, instead of the living organisms.

When one remembers that slight variations in the technic of staining may cause the nucleus of *E. coli* to resemble so closely that of *E. histolytica* that the most expert protozoologists have been misled and have diagnosed one for the other, it will be easily understood how difficult it is for those having but a limited experience to differentiate species by the use of stained preparations.

The fact remains, that after over ten years of study of the intestinal entamebas by numerous protozoologists, we are now, so far as species are concerned, just about where we were immediately after the publication of Schaudinn's paper. While many new species have been described, and additions, important from a morphological point of view, have been made to our knowledge, we must admit that, at present, only two species of entameba have been actually proved to exist in the intestine of man, and these are the two species described by Schaudinn. We have heard, to adapt Omar:

> great argument
> About it and about: but, evermore,
> Came out by the same door as in we went.

It is true, however, that in properly stained preparations there are generally distinct differences in the appearance of the nucleus of *E. histolytica* and *E. coli*, that are sufficient to allow the experienced observer to distinguish them; while the cysts are quite characteristic for each species. Moreover, it is possible to differentiate them in the living condition, and this is undoubtedly the preferable method for the clinician. It should be understood that the morphology of these parasites varies greatly with the character of the feces containing them, and, therefore, with the clinical character of the infection, in the case of *E. histolytica*. The morphology of the latter species in cases of acute dysentery is quite different from that present in the subacute and chronic infections, so that it becomes necessary to describe the morphology of these parasites as related to the clinical course of the infection.

For convenience of description I shall consider the differentiation of *E. histolytica* and *E. coli* during the vegetative, precystic and cystic stages of development, in both living and stained preparations, indicating in the description the clinical character of the infection present at each stage of development, in the case of *E. histolytica*.

THE DIFFERENTIATION OF E. HISTOLYTICA AND E. COLI IN THE VEGETATIVE STAGE OF DEVELOPMENT

The morphology of these parasites varies greatly during the vegetative stage of development, being most typical when the feces are liquid in character, and, in the case of *E. histolytica,* when the most acute symptoms of dysentery are present. When the feces are semisolid in consistence, as in acute dysentery improving under appropriate treatment, the morphology of this species is that described for the vegetative stage of *tetragena* or the so-called precystic stage; while in the acute exacerbations that occur in chronic entamebic dysentery, the nucleus of *E. histolytica* is typical of the *tetragena* type of nucleus.

DIFFERENTIATION OF THE LIVING ENTAMEBAS

As I have stated, *E. histolytica* and *E. coli* may be distinguished in the living condition and this will be found the best method in ordinary practice.

Size.—It is impossible to differentiate the two species from the size of the organisms, for while *E. histolytica,* in the vegetative stage of development, is generally larger than *E. coli,* individuals of the latter species are observed that are fully as large as the average *histolytica,* and in the subacute types of dysentery *E. histolytica* may even average smaller than *E. coli.* It is when acute symptoms of dysentery are present that *E. histolytica* attains its largest size, measuring sometimes as much as 80 microns in the long diameter, when moving, but the average measurement is from 25 to 40 microns in diameter, when motionless. The measurement of *E. coli* is from 15 to 50 microns in diameter, but, as a rule, this species does not exceed 25 microns, although I have observed specimens measuring as much as 60 microns in diameter.

Shape.—*E. histolytica* is much more actively motile than *E. coli,* and, for this reason, varies more in shape when observed in freshly passed specimens of feces. While the latter is generally spherical or irregularly oval in shape, *E. histolytica* may be much elongated, finger-shaped, or very irregular, owing to its marked motility. Shape is of very little value, however, in the differentiation of the two species.

Motility.—In freshly voided feces *E. histolytica* is very actively motile when compared with *E. coli,* and this is a most valuable means of distinguishing them under the condition mentioned, for I have never observed a specimen of the latter species that approached in motility the commonly observed specimens of *E. histolytica.* In *histolytica,* motion is generally progressive in character, while in *coli* it often consists only in the sending out and withdrawal of pseudopodia unaccompanied by progressive motility. Even when progressive motion is observed in

E. coli it is sluggish in character, and very easily distinguished from the motion of *E. histolytica* when the two species are observed side by side. In my paper published in 1905[18] I called attention to the great value of motility in distinguishing between these two parasites, and in 1908[19] stated that "the motility alone will serve to distinguish these organisms, if both be observed in freshly voided feces; for it will invariably be found that the amebas exhibiting the most active form of motility will present morphological features which show that they belong to the pathogenic species." This opinion I still hold, and it is now held by most investigators who have carefully studied the living organisms.

Cytoplasm.—The cytoplasm of *E. histolytica* is divided into two well-defined portions, the ectoplasm and the endoplasm. The distinction between the two is most marked when the parasite is moving, the glass-like ectoplasm in the advancing pseudopodium being very clearly defined from the more granular and grayish endoplasm, but even in the motionless entameba there is frequently visible a narrow rim of clear ectoplasm. The ectoplasm is hyaline and glass-like in appearance while the endoplasm is granular or alveolar in structure and much less refractile. Vacuoles are often absent in organisms observed in freshly voided feces, but appear in the cytoplasm as the feces become cold. The clear, hyaline, homogeneous-appearing ectoplasm of this species is very characteristic and furnishes one of the most valuable differential features between it and *E. coli,* as they are observed in the living condition.

The cytoplasm in *E. coli* is also divided into an ectoplasm and endoplasm, but the two portions are poorly defined when compared with *E. histolytica.* The ectoplasm of *E. coli,* while distinguishable in the moving organism, does not present the clear glass-like appearance of that of *E. histolytica,* but approaches very closely to the endoplasm in appearance, and gradually merges with it, instead of presenting the well-marked line of demarcation between the two portions almost always observed in *histolytica.* In most instances the endoplasm of *E. coli* contains one or more vacuoles, although not infrequently specimens are observed that are free from vacuoles.

To one who has had considerable experience in the study of these parasites in the living condition, the differences in the appearance of the ectoplasm and endoplasm are alone sufficient to distinguish the two species.

Phagocytosis of Erythrocytes.—In my early papers[20] I called attention to the facts that *E. histolytica* was actively phagocytic for red blood-

18. Craig, Charles F.: Am. Med., 1905, ix, 854, 897, 937.
19. Craig, Charles F.: Jour. Infect. Dis., 1908, v, 324.
20. Craig, Charles F.: Am. Med., 1905, ix, 854, 897, 937; Internat. Clin., 1905, Series 14, iv, p. 242; Jour. Infect. Dis., 1908, v, 324.

corpuscles, while *E. coli* was only rarely observed to contain them, and that this was a very valuable means of distinguishing the two species. This observation has been confirmed by almost every investigator who has studied these parasites in the living condition, and while the absence of erythrocytes from the cytoplasm of an entameba is of no diagnostic importance, their presence in any number in an entameba observed in feces is absolute proof that we are dealing with *E. histolytica*. In feces containing blood, specimens of *histolytica* are frequently observed containing erythrocytes, sometimes being absolutely filled with them, while in the same feces, specimens of *E. coli* are generally entirely free from erythrocytes, and, at most, contain but one or two. This fact is very useful in differentiating the species, and James, in an unpublished article, the manuscript of which he kindly sent me, says, regarding the phagocytosis of erythrocytes by these parasites:

> In mixed infections I have often observed erythrocytes in varying quantities in the interior of the pathogenic organisms, but I found them rarely within the coincident *E. coli*, and then only one or two at a time. Absence of erythrocytes from the endoplasm is not of any particular value in differentiation, but their presence in any quantity is a certain indication of a pathogenic species.

It will thus be seen that James, who has had an extensive experience in the study of these parasites on the Isthmus of Panama, confirms all that I have previously claimed regarding the importance of this phenomenon in the entameba. A good clinical rule to follow, therefore, is that an entameba occurring in a dysenteric stool and containing erythrocytes should be regarded as *E. histolytica*.

The Nucleus.—The characteristics of size, shape and division into ectoplasm and endoplasm are similar for *E. histolytica* as observed in the feces from acute dysentery cases and during the acute exacerbations in the chronic stage of the disease, but this is not true of the appearance of the nucleus, in either fresh or stained preparations. It was the nonrecognition of this fact that led to the description of the so-called *E. tetragena* as a new species, when, in reality, one stage in the development of *E. histolytica* was being observed. We now know that the nuclear structure of this species may vary, at various stages in the life-history, from that typical of *histolytica*, as described by Schaudinn, to that typical of *tetragena*, as described by Viereck and Hartmann, this variation being accompanied by definite changes in the clinical picture of infection with this species. When the disease is in the most acute stage, *E. histolytica* presents the typical *histolytica* type of nucleus, while in the subacute stage, and during the acute exacerbations of the infection in chronic cases, the *tetragena* type of nucleus is present. As the latter type of nucleus approaches that of *E. coli* morphologically, it is most important to differentiate it from the latter, and this is possible

in stained preparations, but in living organisms the differentiation of the two species must be made on other features than the structure of the nucleus, when the *tetragena* type is present.

In acute cases of dysentery the nucleus of *E. histolytica* is usually invisible, and, when visible, consists only of a very delicate, refractile ring, representing the nuclear membrane, containing within it one or more refractile granules, representing the chromatin. In the vast majority of instances the nucleus of this species is invisible in cases presenting acute symptoms of dysentery, thus distinguishing it from *E. coli*, in which the nucleus is almost invariably visible in the living parasite.

The nucleus of *E. coli* consists of a well-defined ring of refractile granules, containing within it either scattered refractile granules, or a refractile mass near the center representing the karyosome. It is much larger than the nucleus of *E. histolytica* and can be seen with but little difficulty in either the motile or the immotile entamebas.

In *E. histolytica* the nucleus is generally situated near the periphery of the organism and changes its position rapidly with the movements of the ectoplasm or endoplasm, while in *E. coli* the nucleus is more central in position, and, owing to the slight motility of this species, it tends to remain stationary in the endoplasm.

In the subacute and early chronic cases of dysentery, however, when the nucleus of *E. histolytica* is of the *tetragena* type, it is also almost always visible, having a well-defined nuclear membrane consisting of a ring of refractile granules, while within it there is considerable chromatin in the form of refractile granules or masses. In my opinion, one would have to be very confident in his ability to differentiate minute morphology to assert that the nucleus of *E. histolytica* could be differentiated from that of *E. coli* in living organisms, when the *tetragena* type of nucleus is present. At this time the differentiation of the two species must rest on other data, as I have stated.

From what has been said it is evident that the differentiation of *E. histolytica* and *E. coli*, in the living condition and during the vegetative stage of existence, depends on several factors, the most important being motility, character of the ectoplasm and the endoplasm, and the phagocytosis of erythrocytes. In making such a differentiation I believe that any entameba occurring in the intestinal tract of man, possessing active motility of progressive character, having a well-differentiated ectoplasm and endoplasm, and containing erythrocytes, may unhesitatinly be diagnosed as *E. histolytica;* while an entameba having but sluggish motility, slight differentiation of the ectoplasm and endoplasm, and no erythrocytes in the endoplasm when the feces contain blood may be correctly regarded as *E. coli*. I myself would not hesitate to make a

diagnosis of *E. histolytica* when the feces contain entamebas with erythrocytes within them, and I believe that this is a safe clinical rule to adopt.

DIFFERENTIATION IN STAINED PREPARATIONS

The differentiation of *E. histolytica* and *E. coli* in stained preparations, during the vegetative stage of development, depends entirely on the morphology of the nucleus and on the presence of erythrocytes within the cytoplasm. Neither shape nor the size, neither the presence or number of vacuoles nor the distinction between ectoplasm and endoplasm is of the least value in the differentiation of the species in preparations stained in the manner described in this contribution.

As in the living specimen, the presence of red blood-corpuscles within the endoplasm is sufficient to differentiate *E. histolytica* from *E. coli*, but very frequently the feces do not contain blood, and in such instances differentiation must depend entirely on the structure of the nucleus. This can be done, in properly stained preparations, by one experienced in the study of the protozoa, but, unless this is true, it is much more safe to rely on the differences in the morphology of the living parasites in differentiating the two species during the vegetative stage of life.

The Nucleus.—In wet-fixed and hematoxylin-stained preparations the nucleus of *E. histolytica* presents two distinct morphological types, the true *histolytica* type (Fig. 1), found in the most acute cases of dysentery, and the *tetragena* type (Fig. 2), found in the subacute or more chronic cases of the disease.

The *histolytica* type of nucleus, the species type, is spherical or oval in shape in the stained preparations, although if pressure has been used in making the smears the nucleus may be much distorted. It is characterized by the very delicate nuclear membrane, stained black, and often not more than a line in thickness. In some instances the membrane is thickened at one portion of the periphery, giving it a crescentic appearance. On the inner side of the nuclear membrane very minute dots of black-stained chromatin may sometimes be observed, while at the center of the nucleus there is a very minute and delicate karyosome, usually composed of a single dot or mass of chromatin. In rare instances a minute granule of chromatin, more deeply stained than the karyosome, may be seen lying within the latter, forming the centriole.

Within the nuclear plasma between the karyosome and the membrane a few minute dots of chromatin may be visible, lying on the nodal points of a faintly stained linin network, but very many organisms are observed in which the nucleus appears to consist only of a delicate nuclear membrane enclosing a minute karyosome, the hyaloplasm being entirely free from chromatin. At this stage of development the amount of nuclear

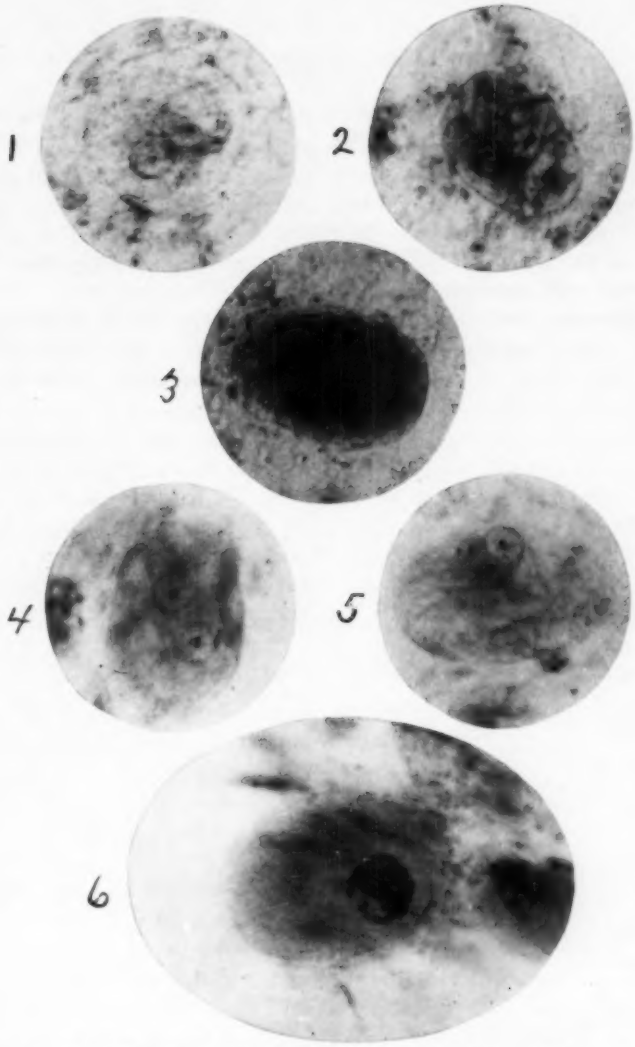

Fig. 1.—Vegetative form (trophozoite) of *E. histolytica*, showing typical *histolytica* type of nucleus; × 1,300. This and the following photomicrographs were taken with a No. 2 eye-piece and a 1/12 oil-immersion objective. All specimens were wet-fixed and stained with iron-hematoxylin. The photomicrographs were taken by Capt. Arthur C. Christy, Army Medical School, Washington, D. C., to whom my thanks are due for many courtesies.

Fig. 2.—Vegetative form of *E. histolytica* showing *tetragena* type of nucleus × 1,300.

Fig. 3.—Vegetative form of *E. histolytica* showing simple division. One nucleus is of the typical *histolytica* type, while the other is of the *tetragena* type; × 1,300.

Fig. 4.—Vegetative form of *E. histolytica*, in which the two nuclei resulting from division are of a modified *tetragena* type; × 1,300.

Fig. 5.—Vegetative form of *E. histolytica* undergoing simple division. The two nuclei are of the *tetragena* type; × 1,300.

Fig. 6.—Vegetative form of *E. coli*. Note clumps of chromatin on nuclear membane and the large compact karysome; × 1,200.

chromatin is much less in *E. histolytica* than in *E. coli*, while the nuclear membrane and karyosome are much more delicate in appearance.

In parasites undergoing simple division two nuclei may be observed, both of the *histolytica* type, although rarely one of the nuclei may be of the *tetragena* type, as shown in Figure 3. In the *histolytica* type of nucleus I have never observed the well-marked cyclical changes so characteristic of the *tetragena* type of nucleus.

The *tetragena* type of nucleus, which is characteristic of *E. histolytica* during certain stages of its development, differs so markedly from the *histolytica* type that for several years entamebas showing this type of nucleus were regarded as belonging to a distinct species. The nuclear membrane, in stained preparations, is of considerable thickness as compared with the membrane of the *histolytica* type and large clumps of chromatin are not infrequently observed on its inner surface. The chromatin may be collected at the center of the nucleus, forming a deeply stained, solid-appearing karyosome closely resembling that of *E. coli*, or more frequently, it has a characteristic arrangement within the nucleus, either being scattered on the inner side of the nuclear membrane or as minute, deeply stained granules on the dimly stained linin network, while the karyosome is comparatively large, irregular or roughly spherical in shape, surrounded by an unstained area, and containing a well-defined centriole. The latter consists of a deeply stained dot or mass of chromatin, sometimes also surrounded by an unstained area.

Certain well-defined cyclic changes, first described by Hartmann, occur in the nucleus of *E. histolytica* when the *tetragena* type of nucleus is present, and these changes are much more prominent in this species than in *E. coli*. The karyosome sometimes appears as a fine network enclosing an unstained area within which lies the centriole, which may show division into two granules; the outer border of the karyosome may be bounded by an unstained area of some extent, at the outer border of which the linin network is observed, the boundary of the clear unstained area being formed of distinct dots of chromatin circularly arranged. Again, the karyosome may stain almost uniformly throughout, the centriole appearing as a more deeply stained central dot, or it may be invisible. The linin network varies in staining qualities, at times being quite distinct while at others it is almost unstainable. Lying on the linin network there are generally numerous dots of chromatin which also vary in their arrangement, while the inner border of the nuclear membrane is covered with minute granules of chromatin, or irregular, larger masses, quite like those occurring commonly on the nuclear membrane of *E. coli*. These changes in the morphology of the nucleus of *E. histolytica* are certainly very characteristic of the species, for while some-

what similar changes also occur in the nucleus of *E. coli*, they are much less striking and offer no difficulty in the differentiation of the species.

In many instances, if one searches carefully, intermediate types of nuclei between the *histolytica* and *tetragena* types may be observed in *E. histolytica*, and organisms are observed containing two nuclei (Fig. 4). These parasites are undergoing simple division and the two nuclei may be of the *histolytica*, *tetragena* (Fig. 5) or intermediate type, or one may be of the *histolytica* type while the other is of the *tetragena* type, as already noted.

In stained specimens degenerative forms of *E. histolytica* are frequently observed, in which the nucleus has either wholly or partially disappeared and the cytoplasm contains threads, granules and blocks of chromatin. These are the forms that have been mistaken in the past for reproduction by gemmation or budding, and that have caused so much confusion in the differentiation of the species. In order to avoid such confusion it is a good working rule to regard any entameba showing chromidia within its cytoplasm as a degenerating organism which should not be considered in distinguishing between *E. histolytica* and *E. coli*.

The nucleus of *E. coli* differs from either the *histolytica* or *tetragena* type of nucleus of *E. histolytica* in being much richer in chromatin, having a thicker nuclear membrane, and a larger and more compact karyosome, in which cyclical changes are not so pronounced. While this is true of the vast majority of entamebas of this species, there do occur specimens of *coli* that have a nucleus very hard to distinguish from the nucleus of *E. histolytica*, when that nucleus is of the *tetragena* type, so that one should study several entamebas in every specimen of feces before arriving at a conclusion regarding the presence or absence of either species.

The nuclear membrane of *E. coli* is quite thick and stains an intense black with hematoxylin. It is even thicker than that of the *tetragena* type of *E. histolytica*, and presents on its inner surface granules or, more frequently, clumps of chromatin (Fig. 6). The karyosome most often appears as a deeply staining mass of chromatin, of comparatively large size, centrally situated within the nucleus, and containing no well-defined centriole. In some instances a centriole may be distinguished as a more deeply staining mass of chromatin near the center of the karyosome. In this species certain cyclical changes also occur in the nucleus and its appearance varies at different times; but they are not so evident as in the *tetragena* type of nucleus of *E. histolytica*. When there is a well-defined and large karyosome there is little evidence of a linin network or of chromatin within it or on the inner surface of the nuclear membrane, but when division of the karyosome occurs the linin network is clearly visible in well-stained specimens, contains much chromatin in the form

of fine granules, and large clumps of chromatin may be observed on the inner side of the nuclear membrane (Fig. 6). The nucleus of *E. coli* is considerably larger than that of *E. histolytica,* in the vegetative stage of development, and, while it may at times resemble the latter in morphology, the thicker nuclear membrane, larger size (Fig. 15), more compact karyosome, and the larger masses of chromatin on the inner side of the nuclear membrane, should serve to distinguish it from any stage in the development of the nucleus of *E. histolytica.*

Specimens of *E. coli* are not infrequently observed undergoing simple division (Fig. 8), and in stained preparations it is possible to study this process from the initial division of the centriole to the complete division of the nucleus. Very frequently organisms are observed in which the karyosome consists of two deeply staining oval masses of chromatin situated near the center of the nucleus or near its periphery; others are observed in which the inner side of the nuclear membrane is covered with large, irregular clumps of chromatin, the karyosome having disappeared; and still others are observed containing two well-developed nuclei possessing the typical *coli* morphology, either situated side by side, or at some distance from one another.

Degenerative forms of this species also are frequent in stained preparations and may cause confusion; but a little study of them should be sufficient to lead to a correct diagnosis regarding their nature.

In summary, the following points are of most assistance in differentiating *E. histolytica* from *E. coli,* in stained preparations, during the vegetative stage of existence:

1. The delicate nuclear membrane and minute karyosome of *E. histolytica,* when the *histolytica* type of nucleus is present.

2. The presence of the loose karyosome, surrounded by an unstained area, and enclosing a well-defined centriole, characteristic of the nucleus of *E. histolytica,* when the *tetragena* type of nucleus is present.

3. The character of the chromatin observed on the inner side of the nuclear membrane in *E. histolytica,* the granules or clumps being smaller and less numerous than in *E. coli.*

4. The presence of erythrocytes within the cytoplasm, characteristic of *E. histolytica.*

The nucleus of *E. coli* is characterized, in stained preparations, by the thick nuclear membrane; the large, compact karyosome in which the centriole is either invisible or ill defined; and by the large clumps of chromatin frequently observed on the inner boundary of the nuclear membrane.

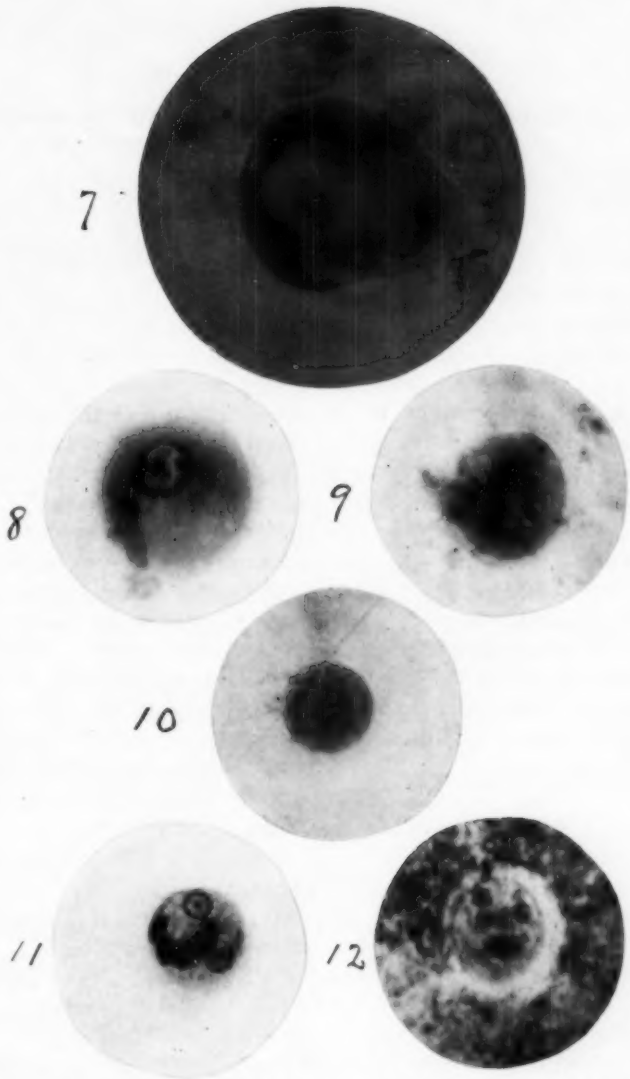

Fig. 7.—Very large cyst of *E. coli*, showing the large vacuole that is frequently present in the cysts before the primary division of the nucleus. Note the spindle-shaped karyosome which is frequently observed just prior to the division of this porton of the nucleus; × 1,200.

Fig. 8.—Young cysts of *E. coli*, showing division of the karyosome and the thick nuclear membrane; × 1,200.

Fig. 9.—Precystic form of *E. coli*. Note reduction in size and the elongation of the karyosome prior to division; × 1,200.

Fig. 10.—Early cystic stage of *E. histolytica*, showing small size and typical nucleus; × 1,150.

Fig. 11.—Encysted form of *E. histolytica*, showing the division of the primary nucleus into two nuclei; also the large chromidial masses characteristic of this species; × 1,150.

Fig. 12.—Encysted form of *E. histolytica*, showing the four daughter nuclei characteristic of the fully developed cyst; also a large chromidial body; × 1,150.

THE DIFFERENTIATION OF E. HISTOLYTICA AND E. COLI IN THE PRECYSTIC STAGE OF DEVELOPMENT

When the symptoms of dysentery are disappearing, that is, in subacute cases, there occurs a stage in the developmental cycle of *E. histolytica* in which the parasite becomes markedly reduced in size (Fig. 10) and the structure of the nucleus tends to assume a phase intermediate between that of the *histolytica* and *tetragena* types. This is the so-called precystic stage of the parasite and, as it occurs when symptoms are slight or practically absent, it is of importance that these entamebas be distinguished from the harmless *E. coli*. Unfortunately, it is just at this stage in their development that it is most difficult to differentiate the two species, and if only these forms be present it is often impossible. Enough typical vegetative or cystic forms are usually present, however, to enable one to make the diagnosis. In my opinion, it is this stage in the development of *E. histolytica* that has been described by Elmassian as *E. minuta*.

Living Preparations.—In the precystic stage of development *E. histolytica* is markedly reduced in size, the average measurement being from 10 to 20 microns in diameter; there is less distinction between the ectoplasm and endoplasm; motility is absent or very sluggish; and a nucleus is generally visible. It will thus be seen that at this stage of development this species is practically indistinguishable from *E. coli*, in the living condition. A careful search will generally reveal the presence of typical cysts or vegetative forms, however, but unless such are present, it must be confessed that it is mere guesswork, in the vast majority of instances, to state that *E. histolytica* is the parasite present.

Stained Preparations.—It is fully as difficult to differentiate the two species in the stained as in the living preparations, when they are in the precystic stage of development. At this time the nucleus of *E. histolytica* is morphologically intermediate in structure between that of the *histolytica* and *tetragena* type, the nuclear membrane being thickened, and the karyosome more compact and uniformly staining, while the centriole is often indistinguishable. All of these changes cause the nucleus of this species to resemble that of *E. coli*, there being a very considerable increase in the amount of nuclear chromatin preparatory to encystment. I believe that it is practically impossible, in the vast majority of instances, to differentiate the two species in stained preparations, at this time; but, as has been said, a careful search of the feces in such infections will generally show a few typical cysts or vegetative entamebas.

Very frequently the cytoplasm of both species contains chromidia in the form of threads, granules or clumps, and it is difficult to be sure whether such organisms are degenerating or whether the appearance of the chromidia is a normal process prior to cyst formation. These forms,

however, may be disregarded as they are of no value in differentiating between the two species.

THE DIFFERENTIATION OF E. HISTOLYTICA AND E. COLI IN THE CYSTIC STAGE OF DEVELOPMENT

When the stools are semiformed or formed, following an attack of entamebic dysentery, or in the intervals between recurrences, *E. histolytica* undergoes encystment, the cyst containing four nuclei (Fig. 12) when fully developed. In formed stools *E. coli* also occurs in the encysted stage of development, the cyst of this species producing eight or more daughter nuclei (Fig. 13), eight being the normal number. As the cysts are the active agents of infection in both species, persons harboring them are efficient "carriers" of the infection, and hence the great importance of their discovery, especially in the prophylaxis of entamebic dysentery, which is caused by *E. histolytica*. Fortunately the cysts of *E. histolytica* and *E. coli* are quite easily recognized and differentiated both when living and when stained, and the routine examination of formed stools for these cysts in every person giving a history of diarrhea or dysentery should become as much a part of diagnostics as the examination of the sputum of persons giving a history of chronic cough. The following are the most characteristic features of the morphology of the cysts in both living and stained preparations.

Living Preparations.—The cysts of both species are spherical or oval in shape, refractive, and present a cyst wall which varies considerably in appearance with the age of the cyst, being delicate and single in outline in the younger cysts, and thicker and having a double outline in the older cysts.

Size.—The cyst of *E. histolytica* varies from 10 to 20 microns in diameter, the average being about 12 microns. The cysts of *E. coli* vary from 10 to 25 microns in diameter, the average being about 15 microns. It will thus be seen that the cysts of *coli* are considerably larger than those of *histolytica*, an important differential point.

Cyst Wall.—The cyst wall of both species, in the newly formed cysts, consists of a delicate membrane about a line in thickness, but in the older cysts the wall becomes much thicker, and generally has a double outline. The double outline is more distinct in the cysts of *E. coli* than in those of *E. histolytica*, but a differentiation of the species cannot be made from the character of the cyst wall.

Cytoplasm.—The appearance of the cytoplasm of the cysts of both species varies also with the age of the cyst. In the younger cysts the cytoplasm appears granular, and, in the case of *E. coli*, not infrequently contains included bodies, as bacteria and yeast-cells, but in the older cysts these have disappeared prior to nuclear division. In the early

stages of nuclear division, before and directly after the primary division of the nucleus, a large vacuole may be present in the cytoplasm in both species, but afterward this vacuole disappears in the developing cysts.

Chromidia.—In the cysts of *E. histolytica* the cytoplasm very frequently contains brightly refractive, spindle-shaped, or irregular masses of chromatin, of large size, which are practically characteristic of the cysts of this species. While somewhat similar chromidial masses may rarely occur in the cysts of *E. coli,* I believe that it is justifiable to make a diagnosis of the presence of the cysts of *E. histolytica,* if these chromidial masses are present within the cyst. In the living organism they are very refractive and appear as shining masses within the cyst.

Nuclei.—The cysts of *E. histolytica,* when fully developed, are characterized by the presence of four nuclei. This number is never exceeded, in my experience, and any specimen of feces containing cysts containing no more than four nuclei may be considered as infected with this species. The nuclei appear as refractive, ring-like bodies within the cytoplasm, and it is generally necessary to focus up and down in order to bring them all into view, as they lie at different levels in the cyst. Any number of nuclei, up to and including four, may be seen in the cysts of *E. histolytica,* but never more than four.

The cysts of *E. coli* contain eight or more nuclei, when fully developed, the normal number being eight, but this is not infrequently exceeded, as cysts of this species may be observed that contain ten (Fig. 14), twelve, fourteen or even sixteen nuclei. The nuclei appear as refractive ring-like bodies, indistinguishable from those of *E. histolytica.* In my experience, any entamebic cyst occurring in the feces of man and containing more than four nuclei is the cyst of *E. coli.*

From the foregoing it is evident that in the living condition the cyst of *E. histolytica* may be distinguished from that of *E. coli* by its smaller size, the presence of the characteristic chromidial masses within the cytoplasm, and of from one to four nuclei. The cyst of *E. coli* is larger, has no chromidial masses, and may contain from one to sixteen nuclei, the normal number being eight.

Stained Preparations.—In the stained preparation, as in the living organism, the cyst of *E. histolytica* is smaller than that of *E. coli,* and the membrane is thinner and does not so often present a double outline. The characteristic differences, however, are the chromidial masses and the number of nuclei. The chromidial masses stain almost black and vary in number, only one or two being present generally. The nuclei stain intensely and are easily distinguished, but as they lie at different levels in the cysts it is necessary to focus carefully in order to be sure of the exact number. The number present, of course, determines the species of the cyst, the cysts of *E. histolytica* containing generally from two to four

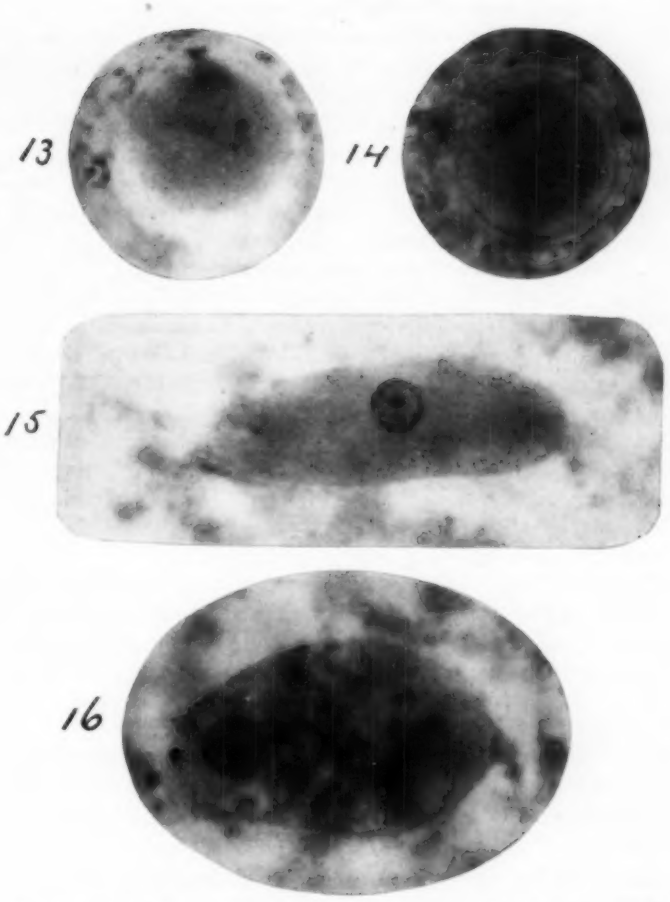

Fig. 13.—Cyst of *E. coli* containing eight nuclei. Only six are visible in the photomicrograph because two lay at a different level in the cytoplasm and thus are out of focus. Note size of cyst in comparison with cyst of *E. histolytica;* × 1,200.

Fig. 14.—Cyst of *Entameba coli* containing ten nuclei. Only five are visible, five being out of focus owing to their position in the cytoplasm. Note large size of cyst; × 1,200.

Fig. 15.—Vegetative form of *E. coli.* Note heavy nuclear membrane and large compact karysome; × 1,200.

Fig. 16.—Vegetative form of *E. coli.* Very large specimen showing typical nucleus; × 1,200.

nuclei, while those of *E. coli* generally contain from two to eight nuclei, this number being sometimes exceeded.

The structure of the nucleus as observed in stained preparations is quite similar, in each species, to that already described as characteristic, and, to one who has had much experience in the examination of these parasites, may alone be sufficient to differentiate the species, but this is best accomplished by attention to the number of nuclei present.

In almost every specimen examined cysts may be observed that are evidently undergoing degeneration, the cytoplasm containing numerous vacuoles and threads and granules of chromatin, while no nuclei are visible. These degenerate cysts have caused much confusion in the classification of entamebas, but are easily recognized and should not be considered in the differentiation of the two species under discussion. The normal cysts always contain well-stained nuclei and show no chromidia in their cytoplasm, with the exception of the large chromidial masses already described as characteristic of *E. histolytica*.

A tabular arrangement of the chief differential points between *E. histolytica* and *E. coli*, in both the living condition and in stained preparations as given below, may prove of service in the diagnosis of the species. It should be remembered, however, as I have stated elsewhere,[21] that "a differential diagnosis of these species does not rest on the presence of a single morphological feature, but should only be made after a careful consideration of all morphological data as well as the life-cycle of the organisms investigated." While this is true, it is also true that there are certain morphological characters that are actually specific, as the presence, in any number, of erythrocytes in the cytoplasm of *E. histolytica*, its four nucleated cyst, and the eight nucleated cyst of *E. coli*. When these are present it is an easy matter to distinguish the two species, but if only the precystic forms can be found, the differentiation is most difficult, requiring frequent examinations and the study of the life-cycle of the parasites.

IMPORTANT POINTS IN THE DIFFERENTIATION OF E. HISTOLYTICA AND E. COLI

VEGETATIVE STAGE OF DEVELOPMENT: LIVING PREPARATIONS

E. histolytica	*E. coli*
Actively motile.	Sluggishly motile.
Ectoplasm glass-like. Clearly differentiated from endoplasm.	Ectoplasm poorly differentiated from endoplasm. Not glass-like in appearance.
Nucleus often indistinct or invisible.	Nucleus visible and distinct.
Erythrocytes present in endoplasm when stools contain blood. Very characteristic.	Erythrocytes absent from endoplasm when stools contain blood.

21. Craig, Charles R.: The Parasitic Amebae of Man, Philadelphia, 1911, p. 195.

VEGETATIVE STAGE OF DEVELOPMENT: STAINED PREPARATIONS

Delicate nuclear membrane and minute karyosome, when *histolytica* type of nucleus is present.	Thick nuclear membrane and large compact karyosome (Fig. 15).
Well-defined karyosome surrounded by an unstained area and containing a centriole, when the *tetragena* type of nucleus is present.	Centriole generally invisible or indistinct.
Smaller granules and clumps of chromatin on inner side of nuclear membrane.	Large granules and clumps of chromatin on inner side of nuclear membrane (Fig. 16).
Cyclical changes very marked in the nucleus when the *tetragena* type is present.	Cyclical changes less marked in nucleus.
Erythrocytes present in endoplasm when stools contain blood.	Erythrocytes absent from endoplasm when stools contain blood.

PRECYSTIC STAGE OF DEVELOPMENT: LIVING AND STAINED PREPARATIONS

In both the living condition and in stained preparations the differentiation of *E. histolytica* and *E. coli* is most difficult in this stage of development, and often impossible. The diagnosis should rest on the character of the vegetative or cystic forms that are almost invariably present together with the precystic forms.

CYSTIC STAGE OF DEVELOPMENT: LIVING AND STAINED PREPARATIONS

E. histolytica	*E. coli*
Cyst smaller.	Cyst larger.
Cyst wall thin. Double outline less frequently observed.	Cyst wall thick. Double outline frequently observed.
Cyst contains characteristic chromidial bodies.	Cyst does not contain chromidial bodies.
Cyst contains from one to four nuclei; never over four.	Cyst contains from one to sixteen nuclei, the normal number being eight when the cyst is fully developed.

In conclusion I would urge the value of the study of the living parasites rather than stained preparations, in the differentiation of these two species of entamebas. I am convinced that for most clinicians this is the best and most practical method of distinguishing them, and that it is only those who have had a very limited experience in the study of the living entamebas who insist on the necessity of staining them in order to differentiate them. The data given in this paper are the result of the study of nearly twelve hundred cases of entamebic dysentery and of hundreds of infections with *E. coli*, and, in almost every case, I think it can be stated that the differentiation of *E. histolytica* and *E. coli* was first made on the living parasites without any special difficulty. In view of this experience I am greatly in favor of the study of living organisms rather than of stained preparations in attempting to distinguish the two species. There is one exception to this rule, and that is in the case of the cysts. I believe that the stained cysts are more readily differentiated than the living cysts, and in the search for "carriers" of these bodies

better results will be obtained if stained preparations are made, as well as living preparations.

The differentiation of the harmless *Entameba coli* from the pathogenic *Entameba histolytica*, and the discovery of "carriers" of the latter species, are not academic questions, but are of the most vital importance in the diagnosis, treatment and prophylaxis of dysentery, and for this reason the microscopical examination of the feces, now so much neglected by the physician, should become a routine measure in the clinical study of every patient in regions where entamebic dysentery occurs.

STUDY XXI: THE IMMEDIATE EFFECT OF REPEATED DOSES OF THEOBROMIN SODIUM SALICYLATE AND THEOCIN ON RENAL FUNCTION IN ACUTE EXPERIMENTAL NEPHRITIS *

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Such authorities as Senator,[1] Osler,[2] Bradford,[3] Herrick[4] and Forschheimer[5] agree that in the treatment of acute nephritis a cautious trial with the use of diuretics may be made with safety, but that such drugs should not be given persistently unless a distinct effect is produced, since large doses or the continued effect of small doses may lead to scanty excretion of urine with retention of urinary products. The experimental evidence for this contention is slight.

Of the diuretics in clinical use, purin derivatives are given most extensively. The exact mechanism by which they produce diuresis is not altogether certain. Munk[6] and Schwartz,[7] from perfusion experiments on the isolated dog's kidney injected with caffein and allied drugs, found a marked diuresis without any evidence of increased flow of blood through the kidney, and concluded that these substances had a specific effect on renal secretory functions. Von Schroeder[8] and Loewi[9] confirmed these observations in normal animals, finding after injections of

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* Submitted for publication Jan. 16, 1914.

* This is one of a series of studies on experimental cardiorenal disease: Study I, Smith: Boston Med. and Surg. Jour., 1908, clviii, 696; Study II, Christian: Boston Med. and Surg. Jour., 1908, clix, 8; Study III, Christian: Jour. Am. Med. Assn., 1909, liii, 1792; Studies IV-XV, Christian, Smith and Walker: THE ARCHIVES INT. MED., 1911, viii, 468-551; Study XVI, Christian and O'Hare: THE ARCHIVES INT. MED., 1913, xi, 517; Studies XVII and XVIII, O'Hare: THE ARCHIVES INT. MED., 1913, xii, 49, 61; Study XIX, Christian and O'Hare: Jour. Med. Research, 1913, xxviii, 227; Study XX, Walker and Dawson: THE ARCHIVES INT. MED., 1913, xii, 171.

1. Senator: Nothnagel's Specielle Pathologie und Therapie, 1896, xix, (1), p. 64.
2. Osler: Practice of Medicine, 1909, p. 691.
3. Bradford: Allbutt and Rolleston's System of Medicine, 1908, iv, 607.
4. Herrick: Osler's Modern Medicine, 1908, vi, 143.
5. Forchheimer: Therapeusis of Internal Diseases, 1913, iv, 58.
6. Munk: Centralbl. f. die med. Wissensch., 1886, xxiv, 481.
7. Schwartz: Arch. f. exper. Path. u. Pharmakol., 1899, xliii, 1.
8. Von Schroeder: Arch. f. exper. Path. u. Pharmakol., 1887, xxii, 39.
9. Loewi: Arch. f. exper. path. u. Pharmakol., 1902, xlviii, 410.

caffein and other purin bodies an increased outflow of urine, salts and urea which they considered to be due to direct stimulation of the epithelium. Phillips and Bradford,[10] and Gottlieb and Magnus[11] supplemented these experiments by oncometer studies. They found usually after injection of diuretics of this group dilatation of the renal blood-vessels which was independent of changes in the aortic blood-pressure and which was accompanied by a marked diuresis; but occasionally diuresis occurred with little vascular dilatation or marked vascular dilatation without any additional outflow of urine. Therefore they believed that while the vasomotor phenomena were important in producing diuresis, more complex processes must be present to explain the effect of purin bodies. Fletcher, Henderson and Loewi[12] made parallel observations and drew identical conclusions. Frey,[13] however, using the oncometer, and Beco and Plumier,[14] working with a perfusion apparatus, found that the amount of urine following the injection of these diuretics was directly related to the quantity of blood going through the kidney in a given unit of time. Hence the evidence on the pharmacological action of the purin diuretics is conflicting. By the use of these drugs, however, in normal animals an increased outflow of urine and its solids occurs whether due to a specific dilating action on the blood-vessels of the kidney or to a stimulation of the renal epithelium or to a combination of both these effects.

Under pathological conditions the kidney's reaction to these drugs is varied. Hellin and Spiro[15] studied the action of caffein in rabbits poisoned with arsenic, aloin, chromium and cantharidin. Diuresis could be obtained in those nephritides in which tubular lesions predominated, but did not occur when the glomeruli were chiefly affected. Weber,[16] working on dogs, found that in chromium nephritis the kidneys were unable to excrete a concentrated urine, so that although sodium chlorid and theophyllin produced an increased outflow of fluid except in most advanced cases, yet there was no increase in the output of the solids of the urine, and in certain animals a retention. Hedinger attacked the problem from a different point of view. Schlayer and Hedinger,[17] and Schlayer and Takayasu[18] showed in experimental nephritis in rabbits that the renal vessels as tested in an oncometer were hypersensitive or hyposensitive to physiological stimuli at different stages of the disease.

10. Phillips and Bradford: Jour. Physiol., 1887, viii, 117.
11. Gottlieb and Magnus: Arch. f. exper. Path. u. Pharmakol., 1901, xlv, 223.
12. Fletcher, Henderson and Loewi: Arch. f. exper. Path. u. Pharmakol., 1905, liii, 15.
13. Frey: Arch. f. d. ges. Physiol. (Pfluger's), 1906, cxv, 175.
14. Beco and Plumier: Jour. de phys. et de path. gén., 1906, viii, 10.
15. Hellin and Spiro: Arch. f. exper. Path. u. Pharmakol., 1896, xxxviii, 368.
16. Weber: Arch. f. exper. Path. u. Pharmakol., 1906, liv, 1.
17. Schlayer and Hedinger: Deutsch. Arch. f. klin. Med., 1907, xc, 1.
18. Schlayer and Takayasu: Deutsch. Arch. f. klin. Med., 1910, xcviii, 17.

While hypersensitive the vessels dilated abnormally with a marked diuresis, while hyposensitive they no longer reacted and oliguria resulted. Hedinger[19] applied this method to animals injected with different renal irritants, using as vascular stimuli theophyllin, digalen and digipuratum in small doses. Diuresis occurred only when the renal vessels were physiologically intact. MacNider[20] was unable to confirm these results entirely. He observed the renal vascular response to caffein, theobromin, digitalen and saline solutions in dogs with uranium nephritis. His animals were divided into two main groups, those anuric or practically anuric with a normal blood-pressure and normal renal vascular system which showed no diuretic reaction to the various drugs, and those with an identical blood-pressure and renal vascular response which, however, had a definite diuresis. In the anuric group, the histological examination showed marked epithelial desquamation which produced an encroachment on the tubular lumen. In the second group this was less distinct. He concluded that mechanical obstruction to the lumen of the tubules played a part in determining the amount of urine. Mosenthal and Schlayer[21] studied the effect of repeated doses of sodium chlorid and caffein on the amount of urine and chlorid excretion and on renal volume as recorded by an oncometer in uranium and chromate nephritis in rabbits. In most advanced cases of each type of lesion neither diuretic was active; but in the early stage of chromate nephritis, sodium chlorid produced an increased outflow of urine and chlorid with renal dilatation, while caffein in repeated doses caused a diminishing amount of these substances with vascular contraction. In the early stage of uranium nephritis directly opposite results were obtained. These findings were explained on the assumption that in one type of lesion the functional condition was hypersensitive to fatigue by caffein, and in the other to fatigue by chlorid. Finally, Christian and O'Hare[22] gave theobromin sodium salicylate, and Walker and Dawson[23] gave theocin, caffein, potassium acetate and spartein sulphate from day to day to rabbits poisoned with uranium nitrate. In most severe cases all these drugs seemed to shorten the animals' lives, but in the less severe cases theobromin sodium salicylate, at least, apparently had a beneficial action. Therefore, from the literature it appears that in mild cases of acute experimental nephritis various diuretics cause an increased output of urine unless repeated rapidly enough to cause renal fatigue, and they are of possible therapeutic benefit. In severe cases, however, they are not pharmacologically active and are even injurious.

19. Hedinger: Deutsch. Arch. f. klin. Med., 1910, c, 305.
20. MacNider: Jour. Pharm. and Exper. Therap., 1912, iii, 423.
21. Mosenthal and Schlayer: Deutsch. Arch. f. klin. Med., 1913, cxi, 217.
22. Christian and O'Hare: The Archives Int. Med., 1913, xi, 517.
23. Walker and Dawson: The Archives Int. Med., 1913, xii, 171.

Except for investigations regarding the total amount of urine and its constituents, no systematic studies have been reported as to the influence of diuretics on renal excretory functions under pathological conditions. This paper describes a series of experiments which were made to determine the immediate effect of repeated doses of theobromin sodium salicylate and theocin on the amount of water, nitrogen, sodium chlorid and phenolsulphonephthalein eliminated during different stages of acute experimental nephritis.

Rabbits were injected subcutaneously with toxic amounts of potassium bichromate or uranium nitrate in sufficient quantity to produce definite changes in renal function without rendering the animals anuric. These drugs were chosen because chromium produces marked tubular destruction in the kidney, leaving the glomeruli practically intact, while uranium nitrate produces lesions in both glomeruli and tubules. Thus renal function was tested under diverse pathological conditions. To obtain different degrees of severity of the same type of nephritis, animals were selected at varying times after injection and renal excretion was studied in the following manner: The animal to be observed was anesthetized by a subcutaneous injection of urethane (ethyl carbamate) in dosage of from 1.25 to 1.50 gm. per kilogram of body-weight. At the same time 2 gm. of urea and 2 gm. of sodium chlorid were administered in 50 c.c. of water by stomach-tube to insure a flow of salt and nitrogen containing fluid. It was realized that these three substances in themselves were powerful diuretics, but it was believed that after their rate of excretion was once established, any marked subsequent variation during the time of these experiments was due to changes in renal function brought about by the drugs introduced. Cannulas were inserted into the bladder and jugular vein according to the usual technic and the elimination of phenolsulphonephthalein, water, salt and nitrogen was followed.

The phenolsulphonephthalein test was slightly modified, but was made on the whole according to Rowntree and Geraghty's[24] method for obtaining constant output of the drug. At the beginning of the experiment, depending on the severity of the clinical condition of the animal, from 0.0015 to 0.006 gm. of the dye in 1 c.c. of saline solution was injected intravenously. After the color appeared, the urine during the next thirty minutes was measured in drops and diluted to 10 or 20 c.c. with distilled water, and the amount of phenolsulphonephthalein in an aliquot part estimated by Rowntree and Geraghty's modification of the Autenrieth-Königsberger colorimeter. Except when only traces were recovered, an amount of phenolsulphonephthalein equal to 10 per cent.

24. Rowntree and Geraghty: THE ARCHIVES INT. MED., 1912, ix, 284.

more than the total amount recovered at this time was reinjected into the vein, to be excreted during the following interval, thus allowing for the loss between observations. In the few animals which were unable to excrete appreciable amounts of the drug, no further injections were made. In this way the phenolsulphonephthalein concentration in the blood and tissues was kept as nearly constant as possible, and any distinct increase or decrease in the excretion was assumed to be due to changes in renal function. At the end of every half hour the total amount of urine, nitrogen and salt excretion for the period was recorded. The nitrogen was quantitated by Kjeldahl's method, and the chlorids by the Lütke-Martius method. From these figures the concentration of nitrogen and salt in one hundred drops of urine was found. After the flow of water and phenolsulphonephthalein was approximately constant for two half-hour periods, theobromin sodium salicylate and theocin in increasing dosage were injected intravenously. As Hedinger[25] had found clinically that the repeated use of small amounts of diuretics often produces greater effect than single large doses, it seemed advisable to determine experimentally the action of repeated small injections of such drugs on renal function. Therefore, solutions of theobromin sodium salicylate[26] and theocin sodium acetate were made containing 10 mg. to the cubic centimeter of the former drug, and 5 mg. to the cubic centimeter of the latter drug (corresponding to 0.3 gm. of theobromin sodium salicylate and 0.15 gm. of theocin if given to a man weighing 150 pounds). At first 1 c.c. of theobromin sodium salicylate solution was given, followed in fifteen minutes by 2 c.c., yielding a total of 30 mg. of the drug. Then 1 c.c. of theocin solution was injected, and at fifteen-minute intervals 2 c.c., 3 c.c. and 4 c.c., making in all 50 mg. of the drug. Finally, the animal was killed and the kidneys were examined histologically after Zenker fixation and eosin-methylene blue staining. The drugs were given in this order and amount for arbitrary reasons. No effort was made to determine their relative efficacy of action. The experiments were conducted solely to discover the immediate effect on renal function in different kinds of acute experimental nephritis of the repeated injection of two typical purin diuretics.

The results have been grouped into three tables, each table representing the mean of eight experiments. While each observation in general showed the same features, by averaging them in this way, any exceptional finding due to individual peculiarity of the animal was ruled out.

Table 1 shows that under the conditions stated for the time observed, the excretion of fluid in normal animals diminished slightly but con-

25. Hedinger: München. med. Wchnschr., 1912, lix, 1098.

26. The substance used in these experiments was the brand of theobromin sodium salicylate made by Knoll & Co., and sold under the proprietary name "Diuretin." The theocin sodium acetate was made by Bayer & Co.

TABLE 1.—EXPERIMENTS ON NORMAL ANIMALS

PART I. NORMAL ANIMALS

Average of Rabbits I, II, III, 737:* Average weight, 1,750 gm. Histological findings negative. Urethane narcosis. At the beginning of the experiment each animal was given 2 gm. of urea and 2 gm. of sodium chlorid in 50 c.c. of water by stomach-tube. Injected intravenously with phenolsulphonephthalein.

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt. Injected	Remarks
		Total No. Mg.	Mg. Per 100 Drops	Total No. Mg.	Mg. Per 100 Drops		
1–30	180	43	24	55	31	48	
35	...	..	..	..	..	..	Reinjected with phthalein.
35–65	175	54	31	48	27	46	
70	...	..	..	..	..	..	Reinjected with phthalein.
70–100	164	72	44	40	24	49	Killed.

* The numbers of these animals correspond to those recorded in the laboratory of Theory and Practice, Harvard Medical School.

PART II. NORMAL ANIMALS WITH DIURETICS

Average of Rabbits III, IV, 731, 737: Average weight, 1,750 gm. Histological findings negative. Urethane narcosis. At the beginning of the experiment each animal was given 2 gm. of urea and 2 gm. of sodium chlorid in 50 c.c. of water by stomach-tube. Injected intravenously with phenolsulphonephthalein.

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt. Injected	Remarks
		Total No. Mg.	Mg. Per 100 Drops	Total No. Mg.	Mg. Per 100 Drops		
1–30	96	47	49	45	46	52	
35	...	...	..	..	..	..	Reinjected with phthalein.
							1 c.c. of theobromin sodium salicylate.
50	...	...	..	..	..	..	2 c.c. of theobromin sodium salicylate.
35–65	150	62	41	52	34	58	
70	...	...	..	..	..	..	Reinjected with phthalein.
							1 c.c. of theocin solution.
85	...	...	..	..	..	..	2 c.c. of theocin solution.
70–100	584	198	34	70	12	66	
105	...	...	..	..	..	..	Reinjected with phthalein.
							3 c.c. of theocin solution.
120	...	...	..	..	..	..	4 c.c. of theocin solution.
105–135	572	188	32	61	10	64	Killed.

TABLE 2.—Uranium Experiments

PART I. ANIMALS WITHOUT DIRECTICS

Average of Rabbit 754: 5 mg. of uranium nitrate subcutaneously; 48-hour nephritis histologically showing marked tubular lesions.

Average of Rabbit 755: 3 mg. of uranium nitrate subcutaneously; 24-hour nephritis histologically showing mild tubular lesions.

Average of Rabbit 756: 5 mg. of uranium nitrate subcutaneously; 72-hour nephritis histologically showing mild tubular lesions.

Average of Rabbit 757: 6 mg. of uranium nitrate subcutaneously in divided doses; 48-hour nephritis histologically showing advanced tubular lesions and slight glomerular lesions.

Average weight, 2,000 gm. Urethane narcosis. At the beginning of the experiment each animal was given 2 gm. of urea and 2 gm. of sodium chlorid in 50 c.c. of water by stomach-tube. Injected intravenously with phenolsulphonephthalein.

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt. Injected	Remarks
		Total No. Mg.	Mg. Per 100 Drops	Total No. Mg.	Mg. Per 100 Drops		
1–30	213	59	28	30	14	21	
35	...	...	..	..	..	..	Reinjected with phthalein.
35–65	184	44	24	32	17	21	
70	...	..	..	..	..	..	Reinjected with phthalein.
70–100	137	29	21	27	20	18	
105	...	..	..	..	..	..	Reinjected with phthalein.
105–135	138	23	17	25	18	20	Killed.

PART II. ANIMALS WITH DIURETICS

Average of Rabbit 741: 3.5 mg. of uranium nitrate subcutaneously; 24-hour nephritis histologically showing mild tubular lesions.

Average of Rabbit 730: 9 mg. of uranium nitrate subcutaneously in divided doses; 120-hour nephritis, histologically showing advanced tubular lesions, and slight glomerular lesions.

Average of Rabbit 728: 4 mg. of uranium nitrate subcutaneously; 48-hour nephritis histologically showing marked tubular lesions.

Average of Rabbit 729: 5 mg. of uranium nitrate subcutaneously in divided doses; 96-hour nephritis, histologically showing marked tubular lesions.

Average weight 1,900 gm. Urethane narcosis. At the beginning of the experiment each animal was given 2 gm. of urea and 2 gm. of sodium chlorid in 50 c.c. of water by stomach-tube. Injected intravenously with phenolsulphonephthalein.

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt. Injected	Remarks
		Total No. Mg.	Mg. Per 100 Drops	Total No. Mg.	Mg. Per 100 Drops		
1–30	176	68	39	18	10	16	
35	...	...	..	..	..	..	Reinjected with phthalein.
							1 c.c. of theobromin sodium salicylate solution.
50	...	...	..	..	..	..	2 c.c. of theobromin sodium salicylate solution.
35–65	332	91	28	32	10	17	
70	...	...	..	..	..	..	Reinjected with phthalein.
							1 c.c. of theocin solution.
85	...	...	..	..	..	..	2 c.c. of theocin solution.
70–100	496	137	28	38	07	18	
105	...	...	..	..	..	..	Reinjected with phthalein.
							3 c.c. of theocin solution.
120	...	...	..	..	..	..	4 c.c. of theocin solution.
105–135	369	97	26	39	11	18	Killed.

stantly, while chlorid and nitrogen excretion varied somewhat both in amount and in concentration without bearing direct relation to the amount of urine. The phenolsulphonephthalein excretion remained almost unchanged. When the diuretics were injected there was a pronounced increase in the amount of urine passed, paralleled by that of sodium chlorid. The concentration of chlorid became lower, suggesting that the excess of salt was carried through the kidney by water. The output of nitrogen was less affected by the diuresis, but increased as did that of the phenolsulphonephthalein, agreeing with Rowntree and Geraghty's[24] observations. On the whole, in normal animals the immediate effect on renal function of repeated injections of theobromin sodium salicylate and theocin was beneficial. This was shown by an increased output of water, chlorid, nitrogen and phenolsulphonephthalein; but by repeated dosage there was evidence of beginning renal fatigue, since the excretion of all these substances diminished during the last period of observation.

As in the first part of Table 1, animals with uranium nephritis which had not received diuretics (Table 2) showed a constant diminution in the amount of urine. In this series the chlorid and nitrogen decreased both in amount and in concentration. The phenolsulphonephthalein remained unchanged. The theobromin sodium salicylate and theocin produced a definite reaction. There was a marked increase in the amount of urine paralleled by that of nitrogen and chlorid without any increase in concentration. The amount of phenolsulphonephthalein, however, did not vary to an appreciable degree. In the last period the evidence of renal fatigue was exaggerated. The total amount of urine and salt diminished to a greater extent than in normal animals, though remaining above the initial level, while the nitrogen and phenolsulphonephthalein were unaffected. Therefore, in uranium animals the immediate effect on renal function of repeated injections of theobromin sodium salicylate and theocin was not harmful. There was an increased output of water, chlorid and nitrogen with an unchanged excretion of phenolsulphonephthalein. The kidney was fatigued more easily than under normal conditions, as shown by the diminishing output of salt and water under the influence of repeated injections of the drugs.

Table 3 shows the effect on animals poisoned with potasium bichromate and treated in similar fashion. As in the previous control experiments, animals without diuretics showed a constant falling off in their salt and water excretions, although the excretion of nitrogen increased without relation to the total amount of urine. The elimination of phenolsulphonephthalein was nearly constant. The action of the diuretics was less marked. While both salt and water were excreted in increased amounts they were proportionately less well put out than in the previous

TABLE 3.—BICHROMATE EXPERIMENTS

PART I. ANIMALS WITHOUT DIURETICS

Average of Rabbit 762: 20 mg. of potassium bichromate subcutaneously; 72-hour nephritis histologically showing mild tubular lesions.

Average of Rabbit 763: 20 mg. of potassium bichromate subcutaneously; 72-hour nephritis histologically showing mild tubular lesions.

Average of Rabbit 764: 40 mg. of potassium bichromate subcutaneously; 96-hour nephritis histologically showing mild tubular lesions.

Average of Rabbit 765: 40 mg. of potassium bichromate subcutaneously; 96-hour nephritis histologically showing well-marked tubular lesions.

Average weight 2,500 gm. Urethane narcosis. At the beginning of the experiment each animal was given 2 gm. of urea and 2 gm. of sodium chlorid in 50 c.c. of water by stomach-tube. Injected intravenously with phenolsulphonephthalein.

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt. Injected	Remarks
		Total No. Mg.	Mg. Per 100 Drops	Total No. Mg.	Mg. Per 100 Drops		
1–30	221	56	25	35	16	45	
35	...	..	..	..	..	..	Reinjected with phthalein.
35–65	219	64	29	38	17	45	
70	...	..	..	..	..	..	Reinjected with phthalein.
70–100	180	54	30	40	22	43	
105	...	..	..	..	..	..	Reinjected with phthalein.*
105–135	180	49	27	41	23	40*	Killed.

* Represents average of three animals. In one animal part of injection was lost.

PART II. ANIMALS WITH DIURETICS

Average of rabbit 732: 20 mg. of potassium bichromate subcutaneously; 24-hour nephritis histologically showing mild tubular lesions.

Average of rabbit 734: 20 mg. of potassium bichromate subcutaneously; 72-hour nephritis histologically showing no lesions, but functionally abnormal.

Average of rabbit 735: 20 mg. of potassium bichromate subcutaneously; 96-hour nephritis histologically showing marked tubular lesions.

Average of rabbit 743: 20 mg. of potassium bichromate subcutaneously; 24-hour nephritis histologically showing mild tubular lesions.

Average weight 1,800 gm. Urethane narcosis. At the beginning of the experiment each animal was given 2 gm. of urea and 2 gm. of sodium chlorid in 50 c.c. of water by stomach-tube. Injected intravenously with phenolsulphonephthalein.

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt. Injected	Remarks
		Total No. Mg.	Mg. Per 100 Drops	Total No. Mg.	Mg. Per 100 Drops		
1–30	192	64	33	40	21	33	
35	...	..	..	..	..	..	Reinjected with phthalein.
							1 c.c. of theobromin sodium salicylate solution.
50	...	..	..	..	..	..	2 c.c. of theobromin sodium salicylate solution.
35–65	224	57	25	40	18	28	
70	...	..	..	..	..	..	Reinjected with phthalein.
							1 c.c. of theocin solution.
85	...	..	..	..	..	..	2 c.c. of theocin solution.
70–100	351	96	27	45	13	24	
105	...	..	..	..	..	..	Reinjected with phthalein.
							3 c.c. of theocin solution.
120	...	..	..	..	..	..	4 c.c. of theocin solution.
105–135	260	64	25	30	11	18	Killed.

series. There was no definite increase in nitrogen excretion, and during the last period a diminution. The most striking feature, however, was the steady decrease in phenolsulphonephthalein elimination during the entire time of observation. In chromate nephritis, therefore, the immediate effect of purin diuretics was to produce a slight diuresis of water and salt, to have little effect on the excretion of nitrogen and to hinder that of phenolsulphonephthalein. If a large enough amount of drugs were given, the kidney was more easily fatigued than in normal or uranium animals, as shown by a greater lessening in the excretion of all the substances studied.

In addition a series of experiments with cantharidin nephritis was attempted. When sufficient amount of the poison was given to produce outspoken lesions, it was found that the animals became anuric and failed to react to the diuretics, confirming the observations of Hellin and Spiro,[15] Schlayer and Hedinger[17] and Schlayer and Takayasu.[18] If lesser amounts were injected, the anatomical and functional conditions of the kidney were so little changed as to make the observations of no value. These experiments, therefore, have been discarded.

On the whole, in normal animals the immediate effect of injections of theobromin sodium salicylate and theocin was to produce an increased outflow of urine, sodium chlorid and to a less extent of nitrogen and phenolsulphonephthalein. After repeated small doses of the drug were given the kidney became fatigued and excreted these substances in lesser amounts.

In animals suffering from uranium nephritis of varying intensity a similar immediate response to these diuretics was obtained except that the kidney became more easily fatigued. In chromium nephritis, on the other hand, while the kidney responded to the theobromin sodium salicylate and theocin by increasing the output of water and chlorid, yet the excretion of nitrogen remained almost constant and that of phenolsulphonephthalein diminished. After large amounts of the drugs had been injected, the signs of renal fatigue were more pronounced than in uranium nephritis or normal animals as the water, chlorid, nitrogen and phenolsulphonephthalein excretions diminished with greater rapidity.

Mosenthal and Schlayer[21] have demonstrated differences in renal fatigability to repeated injections of caffein and sodium chlorid in uranium and chromate nephritis. In these experiments following repeated injections of purin diuretics the kidneys became more easily fatigued in relation to excretion of the substances studied in chromate nephritis than in uranium nephritis or normally. Thus these observations are to a certain extent confirmatory of previous work.

Folin, Karsner and Denis[27] have shown that the non-protein nitrogen of the blood of animals having acute toxic nephritis accumulated in proportion to the severity of the disease. It was most pronounced in uranium and cantharidin nephritis and least marked in chromate nephritis. Clinically, it has long been recognized as a grave prognostic sign in kidney disease. Frothingham, Fitz, Folin and Denis,[28] working with rabbits poisoned with uranium nitrate, showed that the curve of nitrogen accumulation in the blood and of the excretion of phenolsulphonephthalein in the urine correspond closely. Therefore, it seems reasonable to conclude that in uranium nephritis the immediate effect of theobromin sodium salicylate and theocin was beneficial, for by the use of these drugs the outflow of water, sodium chlorid and nitrogen was increased, thereby tending to prevent nitrogenous accumulation in the blood. In chromate nephritis, however, the immediate effect of these diuretics was harmful, since, although they increased the outflow of water and chlorid, the nitrogen excretion was unaffected and the phenolsulphonephthalein elimination was diminished. In each nephritis repeated injections of theobromin sodium salicylate and theocin to the point of fatigue of the kidney was harmful to the renal function by producing a diminution in the total amount of urine, chlorid, and to a less degree, of phenolsulphonephthalein and nitrogen excreted.

CONCLUSIONS

1. The immediate effect of repeated small doses of theobromin sodium salicylate and theocin on renal function in acute experimental uranium nephritis of different degrees of severity is beneficial since they cause an increased output of urine, chlorid and nitrogen and do not diminish the excretion of phenolsulphonephthalein.

2. The immediate effect of repeated small doses of theobromin sodium salicylate and theocin on renal function in acute experimental bichromate nephritis of different degrees of intensity is harmful, since although an increased output of urine and chlorid occurs, the excretion of nitrogen is unaffected and that of phenolsulphonephthalein is diminished.

3. The repeated injection of small doses of theobromin sodium salicylate and theocin in sufficient amount produces a condition of renal fatigue in which the kidney is no longer able to react. This is most easily obtained in bichromate nephritis.

4. Various types of acute experimental nephritis respond differently to repeated injections of theobromin sodium salicylate and theocin both in regard to immediate changes in the excretion of chlorid, nitrogen and

27. Folin, Karsner and Denis: Jour. Exper. Med., 1912, xvi, 789.
28. Frothingham, Fitz, Folin and Denis: THE ARCHIVES INT. MED., 1913, xii, 245.

phenolsulphonephthalein and in regard to the degree of renal fatigue which is induced.

5. These experiments do not include observations on nephritis of the most extreme grade of severity. In the few studies made on animals which had the different kinds of lesions and were anuric, the diuretics were not active in provoking an increased output of either water, chlorid, nitrogen or phenolsulphonephthalein.

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THREE CASES OF ADAMS-STOKES SYNDROME WITH HISTOLOGICAL FINDINGS*

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Although it is established that a complete transverse lesion of the stem of the auriculoventricular conducting system or of its two main branches results in auriculoventricular dissociation, and although a few years ago the problem of heart-block seemed almost settled by the discovery of lesions in the conducting system, it is now apparent that the solution of this problem is not so simple as it appeared at that time. There are cases on record in which heart-block was proved beyond a doubt, but still no lesions in the hearts adequately explaining the failure of the conduction of impulses from auricle to ventricle were to be found.†

The three cases to be presented show three very different histological conditions in the conducting system; and it is somewhat unfortunate that the clinical data do not give us such definite information of the mechanism of the heart as can be positively correlated with these post-mortem findings.

Three hearts from patients with a clinical history suggesting heart-block were kindly entrusted to us for microscopic examination; two by Dr. MacCallum and one by Dr. Bishop.

HEART 1.—*Case Report.*—The case report has been given by Dr. Bishop.[1] The patient, a watchman, aged 75, was admitted to Lincoln Hospital, Oct. 22, 1908. His family history was negative. He had had measles, whooping-cough and small-pox when a child. Later in life he had had muscular rheumatism and a sunstroke. He had drunk whisky moderately. Syphilitic history was denied. The patient was described as having a slow pulse, vertiginous and syncopal attacks and rapid venous pulse. The radial artery was not thickened, even to the degree that would be expected in a patient of his age. A polygraphic tracing was published in the original article.

Macroscopic Examination of the Heart.—The post-mortem examination of the heart was made by Dr. Larkin.[2] "The heart showed a calcareous nodule, situated precisely in the path of the bundle of His. This nodule was evidently the cause of the complete heart-block observed during life."

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* From the Pathological Laboratory of Columbia University, at the College of Physicians and Surgeons, New York City; read in abstract before the New York Pathological Society, Nov. 12, 1913.

† A. F. Stanley Kent has described in detail another connection between the auricle and ventricle beside the bundle of His, situate at the right lateral border of the human heart. (Proc. Roy. Soc., London, 1914, Series B., lxxxvii, No. B594, Biological Sciences, p. 198.)

1. Bishop: Am. Jour. Med. Sc., 1910, cxxxix, 62.

2. Bishop and Larkin: Proc. New York Path. Soc., 1909, New Series IX, 58.

The heart was received from Dr. Bishop in formaldehyd solution and the measurements of the preserved heart revealed nothing of interest.

The edges of both mitral cusps were shrunken and deformed and one could feel a hard calcareous deposit all along the attachment of the posterior cusp. The aortic cusp of the mitral valve was thickened throughout and contained irregular, rough, hard calcareous deposits. This calcareous mass was continuous with that described above in the upper portion of the interventricular septum and in the space between the anterior and right posterior cusps of the aortic valve, that is, directly in the normal pathway of the bundle of His. The cusps of the aortic valve were markedly thickened. The wall of the aorta itself showed patches of atheroma. The coronary arteries, both right and left, presented a very marked sclerosis with patches of atheroma.

Microscopic Examination.—Such a portion of the heart was removed for microscopic examination as would include the calcareous mass which seemed macroscopically to be situated precisely in the path of the bundle of His; namely, such a portion of the septum between the two halves of the heart as included the node, stem, and the branching of the auriculoventricular system. The upper margin of this block ran along the upper edges of the aortic leaflets, and the lower margin parallel with this 3 cm. below the lowest part of the pars membranacea septi.

This piece of tissue was decalcified with nitric acid, embedded in paraffin, cut from above down, parallel with the upper margin, in sections 15 to 19 microns thick. Altogether 2520 sections were cut and for a preliminary study every fortieth mounted. Finally about every fourth section from Section 1322 (where the node was first seen) to 1618 (well beyond the break in the continuity of the auriculoventricular system), and about every tenth from 1618 to 2440, was mounted. All the sections were stained with Weigert's iron hematoxylin and van Gieson's picric acid fuchsin solution except Sections 1392, which was stained with hematoxylin and eosin, and 1334, which was stained by Roehl's method for calcium.

The auriculonodal connections are attenuated (especially the strand to the left) but are uninterrupted. A very few round cells and still fewer polymorphonuclear cells are found at this junction and in the node itself.

The node lies, as usual, to the right of the central fibrous body, but is peculiar in that it is largely cut off from the auricular musculature by fatty tissue. It consists of a network of pale, fine muscle-fibers with crowded nuclei. The nodal artery shows increase of connective tissue in the intima and media; indeed there is sclerosis of the vessels throughout the whole of all the sections.

The node and stem are continuous. The stem is characterized by a greater vascularity, a more marked cellular infiltration and by the presence of more connective tissue than the node, which characters are so marked in the auriculoventricular system at and near the bifurcation of the stem as undoubtedly to be beyond normal limits. The stem progresses normally through the membranous septum in a short course and comes to lie under the endocardium of the left ventricle, when abruptly at its forward end it abuts on a calcified necrotic sphere there bifurcating to form what were judged to be stumps of the right and left branches. The calcified sphere, which lies directly across the path of the stem at its bifurcation, stretches from the endocardium of the left ventricle almost to that of the right.

This calcified sphere forms part of an area of calcification which begins just below the anterior aortic (coronary) cusp and involves the stump of the mitral valve, the connective tissue of the auriculoventricular ring and the undefended space, and the surrounding musculature, namely, that of the left auricle and of the basal part of the interventricular septum and of the auriculoventricular bundle. The area consists in the main of nodules of calcified necrotic material well encapsulated by connective tissue sheaths. In and immediately about some of the encapsulated nodules there is increased vascularity accompanied by pus

cells (mostly round cells but not a few polymorphonuclears). There is also here and there the typical invasion of the calcific nodules by young connective tissue with an occasional giant cell.

At one level, one of these calcific nodules appears to the right of the ventricular end of the stem abutting on its narrowest part, and comes to lie in an indentation of the stem giving the appearance of compressing it. This occurs not far from the bifurcation, namely, at the forward end of the stem, which is composed mostly of thin-walled engorged blood-vessels and connective tissue and proportionately very few muscle-fibers. The undoubted increase in connective-tissue elements and vascularization which characterizes the ventricular portion of the stem also exists in the stumps of the right and left branches.

The stumps just mentioned are, judging by their position, probably the true branches and not the result of a mere splitting of the stem owing to pathological conditions. The lesion in this angle is, as has already been mentioned, a large calcific necrotic sphere which separates the stem and stumps from the rest of the right and left branches proper (Figure 1). The left branch proper is easily identified, but the identification of the continuation of the right branch we cannot assert with the same conviction.

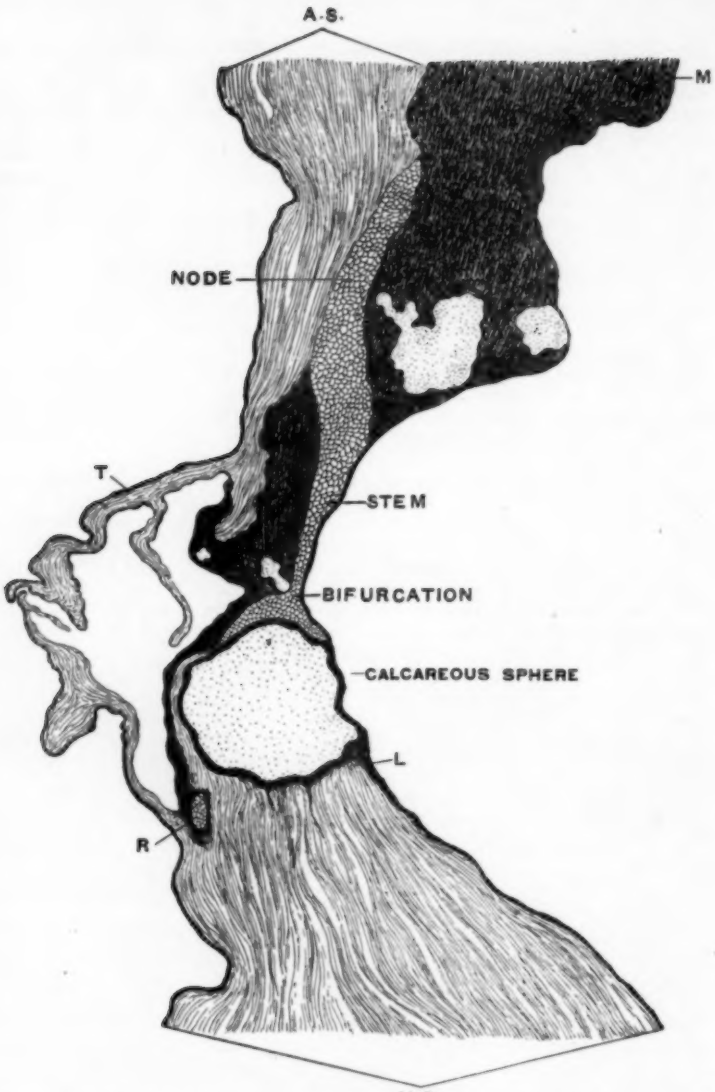
Even on a naked-eye study of the sections it is clear that as one goes from the aortic valve toward the apex of the heart the calcific, necrotic focus involves a more and more extensive area and compromises the auriculoventricular system more and more. And microscopic study reveals that in Section 1462 there is no viable muscle-fiber in the stump of the right branch and that in Section 1480 there is not even an indication of the stump of the right branch,[3] that in Section 1502 there are no muscle-fibers in the stump of the left branch, and that there is in the region of Section 1544 from above down a hiatus of about 1 mm. (1.026 mm.) in the stem where there is no viable musculature. At this level the stem region consists of vascularized connective tissue with much cellular infiltration (mostly round cells, an occasional polynuclear, and a few cells larger than round cells characterized by more cytoplasm and a less compact nucleus).

To summarize, there are for a short distance (examining from above down) no muscle-fibers in the stem. Above this hiatus a large calcific necrotic sphere intervenes between the stem at its bifurcation on the one hand and the right and left branches on the other, and it is only below this hiatus that one branch (namely, the left) is continuous with the body of the stem. In brief, the right and left branches are cut off from the auriculonodal junction because necrotic tissue is situated in the path of the auriculoventricular bundle.

The two branches, cut off as they are from the node, present no abnormalities in their structure.

It should be borne in mind that the identification of the "main" part of the right branch as it descends downward is not absolute, since it is nowhere continuous with the stem, and was judged by its location, the lack of density of its muscle-fibers and its isolation. Farther up, the muscle-fibers of the right limb are slightly finer than those of the surrounding musculature, farther down, slightly coarser with occasionally a clear central space. At one level a small group of muscle-fibers which lies between the right branch and the epicardium presents great resemblances to the right branch, some of its muscle-fibers have even a clear central space so that this segment of musculature is indistinguish-

3. In spite of the absolute break in the continuity of the right branch, impulses may yet have passed from the stump of the right limb through the ordinary ventricular musculature to the ventricular chamber, for it is not unlikely, judging by the anatomical findings in the hearts which we have studied and by physiological considerations, that the auriculoventricular system is not completely isolated between its two ends, namely, all along the line from the auriculonodal connection to the Purkinje fiber-ventricular junction.



Projection drawing of Sections 1438 and 1442, Case 1.—Showing an old calcareous focus, surrounded by connective tissue, which causes complete interruption of the bundle of His. At the bifurcation of the stem a calcific nodule (larger in the following sections) seems to press in upon it. This indented region of the body of the stem and the stumps of its two limbs present manifestly more thin-walled blood vessels and more connective tissue than is normal. At a lower level the necrosis encroaches on the stem to such an extent that it is entirely lacking in muscle-fibers for about 1 mm. from above down. Fibrous tissue is dark; necrotic material is dotted; *A-V* system is honeycombed; *A-S*, auricular septum; *L*, region where isolated left branch of *A-V* system appears in sections lower down; *M*, mitral valve; *R*, isolated right branch; *T*, tricuspid valve; *V-S*, ventricular septum.

able from the right branch as to its structure. Now and again the right limb presents more connective tissue than is found in the neighboring myocardium.

The left branch is characterized by somewhat more connective tissue and is on the whole somewhat paler staining than the surrounding musculature, with its individual muscle-fibers slightly coarser and not so densely constructed, though only rarely presenting a clear central space.

The vessels of both the specialized and typical musculature present sclerosis of the intimal and mesial coats. The muscles about the vessels (especially perhaps those of the outer coat of longitudinal fibers)[4] are finer and paler than the cross-striated typical cardiac muscle-fibers and resemble those of the nodal musculature of the heart. The capillaries are everywhere engorged and in places small extravasations have occurred into the tissues, possibly signifying nothing more than ante-mortem or post-mortem stasis.

There is a good deal of connective tissue in the interventricular septum but no evidence of recent inflammation. Besides the calcified area in the upper part of the interventricular septum (in the angle of bifurcation of the auriculoventricular system and crowding in on the stem) there are isolated areas with lime deposit in a region of much connective tissue between the right and left sides of the interventricular septum and in some connective tissue of the right ventricular musculature.

The smooth muscle of the left endocardium shows more connective tissue than normally. As usual, its muscle-fibers, like those about the vessels, resemble the muscle-fibers of the nodes of the heart.

The endocardium is normal, except that part of the left endocardium which covers the degenerated area where the cellular infiltration extends into it and dense connective tissue replaces the usual loose subendothelial tissue to quite an extent.

The epicardium of the posterior surface (the only epicardium on the sections) presents a thick, compact connective-tissue layer under a not inconsiderable part of the epithelium. In this layer there is (below the level of the node and stem) both a scattered and a dense cellular infiltration; that is, round cells, a few cells with larger cell body and larger vesicular nucleus, and an occasional polynuclear are found scattered and in masses. A most imposing focus of dense cellular infiltration is seen in the subepicardial fatty tissue immediately under the compact connective tissue layer in the lowest sections.

SUMMARY

Though the auriculonodal junction is not very powerful, it is not, apparently, inadequate, but there is an actual interruption of the continuity of the auriculoventricular structure along its course to account for the symptoms in this case; both branches of the conducting apparatus are cut off from the main stem of the auriculoventricular system and from each other. There is undoubtedly more connective tissue in the stem and more vascularity (with its attendant cellular infiltration) than is normal; and the upper ends of both limbs almost wholly and the body of the stem for about 1 mm. of its length have been demolished by a chronic, necrosing, calcifying process. In addition, there is an inconspicuous cellular infiltration at the node, as well as a more

4. This may be due to the direction in which they are cut. It should be noted that in this (as well as in other hearts) some of the ordinary cardiac musculature seems paler or more vacuolated than the rest owing to the direction in which the muscle-fibers are cut.

marked one of the stem and an imposing accumulation of round cells in the subepicardium; there is sclerosis throughout the coronaries and their intermuscular branches, and an increased amount of connective tissue in the ordinary ventricular musculature and some little calcific necrotic tissue.

HEART 2

HEART 2.—*Case Report.*—The heart was forwarded to Dr. L. F. Barker of Johns Hopkins Hospital by Dr. D. A. Campbell, professor of medicine, Halifax, Nova Scotia, together with the following notes: "I first saw J. C., a retired mariner, in the summer of 1905, shortly after he was admitted to an institution for the care of the aged. He was then in his ninety-fifth year. His bodily health was excellent. He was quite corpulent, but unusually active. He was generally cheery and bright in disposition. His mental faculties were well preserved. Physical examinations disclosed the existence of some enlargement of the heart, and the presence of a very loud, harsh, aortic systolic murmur. The pulse rate was 60 and quite regular. During the following winter he had a sharp attack of influenza, from which he recovered completely. During the summer of 1906 he was ill the greater part of the time, but never wholly confined to bed. The most pronounced symptoms were: mental depression, cyanosis, bradycardia (the pulse-rate varying from 10 to 40 without any irregularity), slight seizures resembling petit mal occurring very frequently some days, and Cheyne-Stokes breathing especially at night. On account of the patient's being so fat, no superficial veins could be noticed in the neck. Various remedies were tried to afford relief without much success. Finally belladonna was given in moderate doses and improvement followed. That belladonna favorably influenced the conditions was evident from the fact that when the remedy was discontinued for ten days or so most of the symptoms returned.

"He continued to keep fairly well for a period of ten months, using the belladonna the greater part of the time.

"Early in August, 1907, the symptoms returned and gradually increased in severity, causing his death a month later. The bradycardia was not so marked as before, 30 being the lowest pulse-rate noted, and the epileptiform seizures were infrequent. The more prominent terminal symptoms were: marked cyanosis, Cheyne-Stokes breathing and total disappearance of the radial pulse and cardiac impulse for periods varying from thirty to sixty seconds in duration. This very unusual modification of the pulse was noted for five days before death, and seemed to be closely connected with Cheyne-Stokes respiration. The pulse usually, but not always, disappeared during the period of dyspnea, returning during the period of apnea. No sound whatever could be heard over the cardiac area when the radial pulsations were absent."

Macroscopic Examination of the Heart.—The heart was received from Dr. MacCallum in formaldehyd solution. Grossly the fixed heart presented no abnormality in the region of the conducting system which would account for heart-block.

Microscopic Examination of the Heart.—For microscopic examination the following blocks of tissue were excised:

1. The region of the sinu-auricular node. This block consisted of the auricular wall on either side of the sulcus terminalis of His extending posteriad as far as the interauricular septum and including a part of the superior and inferior venae cavae, and extending anteriad so as to include some of the pectinate musculature (a strip of about 0.5 cm.). Moreover, this block extended along both sides of the crest of the right auricle, thus including not only the part already described on the lateral aspect of the auricle on either side of the sulcus, but also the auricular wall on the median aspect facing the aorta. On the lateral aspect the area reached almost to the auriculoventricular groove; on the median face it

extended from the crest down for about 1 cm. toward the insertion of the aorta and was a continuation, as regards its width, of the lateral portion.

2. The auriculoventricular septum, containing the auriculoventricular node, the main stem, and the beginning of the branches. The sections were cut horizontally, approximately parallel to the stem.

3. A portion of the upper ventricular septum (left side) containing a part of the left branch of the bundle of His.

The first two pieces were embedded in celloidin-paraffin and cut in sections 15 microns thick. Every fifth section was mounted and stained with Weigert's iron-hematoxylin and van Gieson's picric acid fuchsin solution. A few sections were stained with orcein. The third piece was frozen, cut, and one section stained with Scharlach R, one section stained with iron-hematoxylin and van Gieson's solution, and finally one with orcein.

Sinu-Auricular Node Region.—The sinu-auricular node is sclerotic. There are small compact masses of connective tissue between the muscle-fibers; there is more connective tissue at the cephalic than at the caudal end of the node, where the muscle-fibers are separated by looser connective tissue instead of by "wads." Even allowing for individual variation within normal limits, the increase of connective tissue in this case is so marked throughout the entire extent of the node that it may be safely stated to be abnormal. No such fibrosis is present in the musculature immediately about the node or in the other portions of the right auricle examined. In the main the nodal fibers themselves may be described as lacking in cross striations. There is, however, the typical gradual transition from nodal into ordinary auricular musculature. The nodal artery, present in this heart, shows sclerosis of the media and a slight endarteritis. Some of the smaller branches too show these changes as do also the artery on its way to the node and some of the vessels among the ordinary cardiac musculature; occasionally a vessel presents obliterating arteritis. Some of the sections of the arteries have an outer layer of longitudinal smooth muscle-fibers arranged in small bundles.[5] Some of the nuclei of the auricular musculature are large and present uneven distribution of the chromatin and sometimes an irregular margin.[6]

Here and there in the pectinate and other ordinary cardiac musculature as well as in the specialized musculature of the Keith-Flack node, a very few cells which resemble lymphocytes are seen. They are slightly larger, have more cytoplasm, and their nuclei are more vesicular, less compact than those of lymphocytes. At one place in the nodal fat there is a small group of these cells. Larger collections of them appear in the epicardium and subepicardium; the largest at the level of the pace-making apparatus. Sometimes they are found about the subepicardial nervous elements in the neighborhood of the node. Now and again a nerve passes from the subepicardium into the sinu-auricular system. Once a ganglion cell was noticed alongside a capillary in the very center of the nodal tissue.

In general the effects of the pathological changes in the node of Keith and Flack are not yet established, and we do not venture on judging the significance of the sclerosis of this structure in this case.

Region of the Auriculoventricular System.—The connections between the auricular musculature and the node of Tawara are scanty. The node in the sections is an irregular semicircle, the diameter of which lies along the central fibrous body, and the circumference of which is almost entirely surrounded by fat tissue, thus practically separating the nodal from the auricular musculature. There is an abnormal amount of fat tissue in the region of the interauricular septum. The node contains very little connective tissue.

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5. Such bundles of longitudinal smooth muscle-fibers are normally found in all hearts.

6. The nuclei of the swollen type mentioned above were described by Dr. A. E. Cohn in Heart, 1912, iv, 24.

Cross-sections of the artery in the specialized musculature present sclerosis of the intima and the media. Some of the very small vessels have a marked diminution of their lumina; but it is impossible to say which of the two inner coats is involved. There is intimal and mesial sclerosis of the arteries in the interauricular and interventricular septa, and of the coronary artery (with atheroma). There is calcific deposit in the wall of the aorta not only near the opening of the right coronary but even nearer to the auriculoventricular system than that.[7]

As was to be expected in sections of a heart from a patient advanced in years, lipochrome granules are seen in the muscles of the auricle, ventricle, and auriculoventricular system. Some of the nuclei of both the auricular and the ventricular muscles were of the "swollen" type already described. The smooth muscle of the left endocardium presents increase of connective tissue as does the ordinary interventricular musculature especially on the left side.

Though the stem is long and slender, there is nowhere a break in the continuity of the auriculoventricular system.

Upper Ventricular Septum.—The piece from the upper part of the septum on the left side of the heart presents an increased amount of connective tissue in the ordinary cardiac musculature but no evidence of recent inflammation. There is also a slight increase in the connective tissue surrounding some of the vessels; all three vessel coats are sclerotic. There is no fatty infiltration of any of the musculature. The endocardium appears to be normal.

SUMMARY

While it cannot be affirmed that the slightness of the auriculonodal junction is the causative factor in this case of Adams-Stokes disease, yet it is of importance in the light of the fact that Armstrong and Mönckeberg[8] reported an exquisite case of lymphangio-endothelioma of the auriculoventricular node in which it was judged that the break between auricular and nodal musculature was the cause of the heart-block.

The condition of the blood-vessels in the aged patient may have been an accessory factor.

We should expect to find some cellular infiltration in arteriosclerotic hearts, and we do not know that there is any relation between the increase of cells in the interstitial tissue of the sinu-auricular node and of some of the ordinary auricular musculature and the clinical symptoms in this case.

It should be recalled that the interventricular septum, especially on the left side of the heart, presents an increased amount of connective tissue, and that some of the nuclei were of the "swollen" type with scattered chromatin and sometimes with irregular margin.

In brief, the most obvious explanation of the symptoms in this case is the auriculonodal break.

7. In another heart (from a patient without data as to heart-block) though calcareous nodules were found grossly in the neighborhood of the bundle of His, microscopically these did not interfere with the continuity of the auriculoventricular system. This heart was kindly given us for examination by Dr. W. G. MacCallum.

8. Armstrong and Mönckeberg: Arch. f. klin. Med., 1911, cii, 144.

HEART 3

HEART 3.—*Clinical History.*—The heart was received from Dr. W. G. MacCallum, who performed the autopsy and gave us the clinical notes furnished him by Dr. M. G. Porter.

The patient was a white man aged 50. There was no history of syphilis; he had not used alcohol to excess. He had always been strong and healthy until eighteen months before exitus, when he had suffered from several attacks of syncope within a few days. Six months before his death he had a similar seizure and since that time he had frequently complained of giddiness on rising and of shortness of breath. Three days before his death he was seized, after violent exertion, with severe pain in his precordium, radiating down both arms. Subsequently he had repeated attacks of syncope and several epileptiform convulsions with attacks of bradycardia between which the pulse rhythm would be normal. The pulse-rate ranged between 110 and 25 beats per minute. He died in one of his seizures. The urine was acid, specific gravity 1.018, trace of albumin, no sugar. On the day previous to his death a distinct pericardial friction rub was heard over the body of the heart. This was again heard about an hour later ("pericarditis epistenocardia").[9]

Autopsy.—The autopsy revealed the cause of death to be rupture of the wall of the heart and consequent hemopericardium. "The pericardium was found to be filled with blood which was partly clotted: in places the clot was fully 1 cm. thick. There was a ragged hole in the anterior wall of the left ventricle about 1 cm. to the left of the vein that corresponds to the interventricular septum. Some blood-clot was adherent to and extended into this ragged opening. The interventricular septum throughout its entire extent was opaque, of a grayish ochre color and was evidently necrotic. This appearance extended to within 1 cm. of the pulmonary orifice where it was sharply marked off from the adjacent normal heart wall. The wall of the left ventricle was greatly hypertrophied, but its tissue was not abnormal.

"The trabeculae were opaque and frequently broken through. In the angle between the wall of the ventricle and the septum was a hole, through which a probe was passed until it extended through the opening on the anterior surface of the heart. Both coronaries were markedly sclerotic. The anterior descending branch of the left coronary artery was completely occluded by a thrombus 3.5 cm. from its orifice. The sequence of events has evidently been: coronary sclerosis, thrombosis of the left descending branch, infarction of the interventricular septum, rupture of the heart wall, hemopericardium."[10]

Grossly, then, the heart presented an infarction of the interventricular septum including the neighborhood of the main stem of the bundle of His and its branches.

Microscopic Examination of the Heart.—The heart was received from Dr. MacCallum in formaldehyd solution. For microscopic examination the following blocks of tissue were excised:

1. The region of the sinu-auricular node as described under Heart 2.

2. The auriculoventricular septum containing the auriculoventricular node, the main stem and the beginning of the branches.

3. The ventricular septum adjacent to Block 2 (just below it) with the posterior papillary muscle of the left side and the underlying ventricular wall and a piece of the apex.

4. A portion of the upper ventricular septum (left side) containing a part of the left branch of the bundle of His.

5. The anterior papillary muscle of the right ventricle with chordae tendineae.

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9. Sternberg: Compt. rend. XVI Cong. internat. méd., Budapest, 1909, Section VI, Méd. Interne, p. 396; Verhandl. d. XXIX Deutsch. Cong. f. inn. Med., 1912, p. 444.

10. MacCallum: Johns Hopkins Hosp. Bull., February, 1908, No. 203, xix, 50.

6. The base of the anterior papillary muscle of the left ventricle and the underlying ventricular wall.

7. A portion of the wall of the left auricle.

Blocks 1, 2 and 4 were embedded, cut, mounted and stained like the corresponding pieces in Heart 2; piece 3 like blocks 1 and 2 above except that only every fortieth section was stained. The remaining blocks of tissue (5, 6 and 7) were embedded in celloidin-paraffin, cut 15 microns thick and two sections of each stained.

Left Auricle (Block 7).—To begin with the least important block of tissue: The epicardium and adjoining fat and muscle of the wall of the left auricle present cellular infiltration (only an occasional polymorphonuclear leukocyte here). The infiltration is most marked in the epicardium and subepicardium; one small focus was seen in the endocardium. There is sclerosis of the media and intima of the vessels.

Region of the Sinu-Auricular Node (Block 1).—Unlike the portions from the interventricular septum, the block of tissue in the region of the sinu-auricular node presents a comparatively slight cellular infiltration. The round cells (only a few polynuclears are to be seen) are present in the epicardium and subepicardium, to a less extent in the nodal tissue, and to still less an extent in the ordinary myocardium. There is practically no invasion of the endocardium. The area near the inferior vena cava is somewhat more involved than the rest of the ordinary cardiac musculature. The most marked infiltration in this block is found in two regions, namely: (1) in the epicardium at the level of the node and (2) about the subepicardial nervous elements and the musculature on the median face of the crest near the interauricular septum (i. e., the region of the synapse of the vagus as described by Marchand and Meyer[11]). There is a negligible increase in the amount of connective tissue in the head of the pacemaking apparatus. The nodal muscle-fibers are much coarser than those of Heart 2 and show cross-striations clearly. There is the usual gradual transition into ordinary cardiac musculature. There are a few pus cells in the media of the nodal artery. The vessels, both of the specialized and typical musculature, present sclerosis of the two inner coats. As usual, some of the sections of the arteries present an outer layer consisting of bundles of longitudinal non-striated muscles. Some of the nuclei of the musculature of this piece of tissue are of the swollen type. At one point the node encloses ordinary musculature. Here and there a nerve-fiber from the subepicardium is seen to enter the nodal structure; and in its very center a ganglion cell was observed.

It does not seem possible that the very slight cellular infiltration of the pacemaking system in this case could account for any decided influence on the functions of the heart.

Region of the Upper Auriculoventricular System (Block 2).—A very few infiltrating cells — for the most part lymphocytic, rarely polymorphonuclear — are seen in the conducting system. The right branch, and the stem (at the level of its division into two branches) and the central fibrous body near by present more pus cells than the node, the upper stem, and the left branch. Close at hand, at the basal end of the ventricular septum is an infected infarct with pus cells in the capillaries and adjoining tissues. There are pus cells elsewhere (everywhere round cells exceed the polynuclears in numbers), namely in the epicardium, subepicardial fat, auricular musculature, endocardium, tricuspid and mitral and aortic valves. But the most pronounced lesion in this block is situated in the typical ventricular musculature *near* the junctional tissue. Here the musculature has taken an unusual brown tint and many of the muscle nuclei seem to have disappeared. Indeed, some of the muscle-fibers themselves have apparently disappeared in their entirety and have been replaced by connective tissue. The

11. Marchand and Meyer: Arch. f. d. ges. Physiol., (Pflueger's), 1912, cxlv, 401.

remaining muscle-fibers are separated by columns and masses of pus cells and are shrunken. The central fibrous body is a little larger than normal. Practically all the vessels present intimal and medial sclerosis; such are the nodal artery, the aorta, the coronary artery, and, in brief, other vessels in the auricular and ventricular septa. Some of the nuclei of this block of tissue are of the swollen type. The smooth muscle of the left endocardium is normal.

Region of the Lower Auriculoventricular System and of the Terminal Arborizations (Blocks 3, 4, 5, 6).—Upper Ventricular Septum: In Block 4, that is, the piece from the upper part of the septum on the left side of the heart, there is histological evidence of the infarction just as there is in the other blocks from the interventricular septum. There is a round-celled and polymorphonuclear infiltration of the endocardium, subendocardium, the region of the left branch and especially of the ordinary ventricular musculature where many of the muscle-fibers are separated from one another by pus cells, are shrunken, and, though still showing cross-striations, are lacking in nuclei. Some of the muscle-fibers have indeed apparently quite disappeared. There is fat in both the region of the specialized and ordinary cardiac muscles.

Region of the Terminal Arborizations.—In Block 3, namely, the ventricular septum immediately below Block 2, no attempt was made to find and trace the right branch of the auriculoventricular system. The uppermost part of the posterior limb of the left branch was identified macroscopically as if for dissection and marked with India ink[12] and so found in the sections under the microscope; but as we did not follow the left branch continuously in serial sections from the stem on, we cannot assert that we have identified it. In this piece three, there is in the ordinary ventricular musculature, including that of the apex, a continuation of the same lesion (an infected infarct) as described for the other pieces from the ventricular septum, namely, pieces two and four. In that portion of the left limb which was marked with India ink there was no cellular infiltration; but ventral (anterior) of this region there is some infiltration of the endocardium and subendocardial musculature where it is not unlikely that the left branch is involved. Similarly there is infiltration of the endocardium and subendocardial musculature in the apex. On the other hand there is no cellular infiltration of the posterior papillary muscle of the left side. Many of the cells under the epicardium and endocardium of the right and left ventricle and of the posterior papillary muscle are broad and pale and vacuolated; some of these may be Purkinje cells, possibly some Purkinje-like because of the direction in which they are cut and others perhaps because of a Purkinje-cell variety of muscle atrophy.[13] Some of the nuclei of the cells are of the swollen type with unevenly distributed chromatin and irregular margins. The coats of the blood-vessels present an increased amount of connective tissue.

The anterior papillary muscle and chordae tendineae of the right side (Block 5) are normal except for some nuclei of the swollen type; the base of the anterior papillary muscle of the left side and underlying ventricular wall (Block 6) present pus cells in the epicardium and subepicardium and sclerosis of the two inner coats of the vessels.

In short, an almost negligible number of lymphocytic and a still lesser number of polymorphonuclear cells have invaded the auriculoventricular system, and that is all; therefore from an anatomical standpoint the conducting mechanism is in a condition to functionate, but from a physiological viewpoint the sclerosis of its vessels and the presence of even but a few pus cells in the junctional tissue

12. According to Nagayo [in Beiter. z. path. Anat. u. z. allg. Path. (Ziegler's) 1909, xlv, 295] the stripes seen macroscopically are due not only to the presence of the branches of the conducting system, but also to bundles of smooth muscle fibers.

13. Cohn and Lewis: Heart, 1912, iv, 24.

and finally the neighboring infected infarct suggest an inefficiency of circulation and an abnormal condition sufficient to account for heart-block.

DISCUSSION

The sclerosis of the coronary arteries and its branches in these three aged patients suggests that an inadequate circulation played a part in the production of the symptoms of Adams-Stokes syndrome in all three.

As was expected in arteriosclerotic hearts, some increase of the cells in the interstitial tissue was observed, but we do not know what may have been its significance in these cases.

If these are three cases of heart-block, as is suggested by the Adams-Stokes syndrome, then they form a series, Case 1 with an absolute break between the stem at its bifurcation and the two limbs right and left, Case 2 with its inadequate auriculonodal junction and Case 3 without a break in the continuity of the auriculoventricular system.

In the first case described, a lesion to account for heart-block was seen; there was complete transverse destruction of the tissue of the bundle of His. In the second case, it is impossible to state to what extent the sclerosis of the sinu-auricular node and the fact that the auriculonodal junction is slight, affected the functions of the heart. And in the other case, though histological investigation brought to light no such lesion of the auriculoventricular system (i. e., partial or complete break in its continuity), as has been frequently regarded the ultimate anatomical basis of the affection, yet the findings are indicative of such a disturbed circulation and abnormal condition in the neighborhood of the conducting apparatus as might profoundly influence its functional capacity.

SUMMARY

1. The hearts of three patients with Adams-Stokes syndrome presented, the first a calcific mass in the region of the auriculoventricular bundle, the second no gross lesion across the conducting system to account for the symptoms, the third infarction of the ventricular septum.

2. The first patient is reported to have denied a syphilitic history, in the second case the past history is not given, and the third patient is said to have had no lues. All three were well along in years with some sclerosis of the vessels and some increase of cells in the interstitial tissues.

3. The most noteworthy lesions in each case were: in the first, an absolute break in the course of the stem of the auriculoventricular system; in the second, the slight auriculonodal junction and a sclerosis of the sinu-auricular node; in the third, an infected infarct of the ordinary ventricular musculature near the junctional tissue.

4. The possibility of insufficient circulation and of a lesion in the ordinary ventricular musculature as agents in the production of heart-block, are suggested.

We are greatly indebted to Dr. W. G. MacCallum and Dr. L. F. Bishop for the privilege of describing these cases and to Dr. A. E. Cohn for his valuable suggestions.

THE INTRAMENINGEAL TREATMENT OF TABES AND CEREBROSPINAL SYPHILIS *

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The treatment of syphilis of the central nervous system has always been unsatisfactory. The cerebrospinal system is a closed one, and the diffusion of drugs from the circulating blood into the cerebrospinal fluid is slow and incomplete. For this reason organisms which have invaded the nervous tissue or meninges remain more or less protected from the drugs which may be present in the vascular system. For a number of years the desirability of the direct application of antisyphilitic drugs has been recognized. Horsley[1] went so far as to advocate the "heroic" treatment of opening into the subdural space and washing out with mercuric chlorid (1:1,000) solution, but this procedure proved too irritating.

With the advent of salvarsan and neosalvarsan, hope was entertained that this therapeutic agent could be injected intraspinally; but investigation showed that it also damaged the tissues. Wechselmann[2] found that small amounts (1 mg.) when injected into the brains of dogs and rabbits at a concentration of 1:500 and 1:1,000 produced convulsions, weakness of the limbs and death in from two to fourteen days; but small doses, from 0.5 to 0.25 mg., in greater dilution (from 1:2,000 to 1:4,000) caused no reaction. Later he reported[3] the intraspinous injection of neosalvarsan in four patients without reaction, giving each from 0.5 mg. to 3 mg. Marinesco[4] reports thirteen cases in which neosalvarsan was given intraspinally, the dose averaging about 5 mg. In two patients no reaction occurred, but in the remainder the reaction was severe; thus, eight patients developed incontinence of urine or retention, three showed weakness of the extremities and in one case anesthesia of the rectum, legs and buttocks occurred. Ellis and Swift[5] have carefully studied the effects of the intraspinous injection of salvarsan and neosalvarsan in monkeys. They injected small quantities and considered the cell-count as an indication of reaction due to irritation.

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* From the Medical Clinic of the City Hospital at Bay View.

1. Horsley, V.: Neurol. Centralbl., 1910, xxiv, 257.

2. Wechselmann, W.: Die Behandlung des Syphilis mit Dioxydiamidoarsenobenzol, "Ehrlich-Hata 606," Berlin, 1911, p. 11.

3. Wechselmann, W.: Deutsch. med. Wchnschr., 1912, xxxviii, 1446.

4. Marinesco: Diatet. u. physik. Therap., 1913, xvii, 194.

5. Swift and Ellis: Jour. Exper. Med., 1913, xviii, 428.

They found that the intraspinal injection of even minute quantities (0.2 mg.) produced profound cellular reactions which persisted over ten days, while 3.2 mg. caused, in one monkey, paralysis of the hind legs, which lasted several months. The injections of neosalvarsan were, on the whole, less severe, but still gave evidence of irritation in doses as small as 0.045 mg. Repeating the injection seemed to increase these effects.

The study of the effects of the intravenous or intramuscular injection of salvarsan brought to light a very important fact. Plaut[6] showed that the serum of patients who have received salvarsan exerts a definite antisyphilitic effect, whereas normal serum has no such activity, and the serum of patients treated with iodids and mercury has very little such power. Meirowsky and Hartman[7] and Gibbs and Calthrop[8] treated cases of syphilis with good results by injecting subcutaneously serum taken from other patients who had received salvarsan. In the same way Henry Alsten[9] successfully treated several cases of frambesia. Ellis and Swift[10] took up the study of this serum and showed that, when taken from the patient within six hours after the salvarsan injection, it inhibits the growth of the *Treponema pallidum*, but if taken before treatment, or from six to twenty-four hours after treatment, there is no inhibition. They also reported beneficial effects from the intraspinal injection of this serum, with very few irritative phenomena.

This spirochetacidal activity of salvarsanized serum was further studied by Gonder,[11] Castelli,[12] and Ellis and Swift,[13] who showed by the use of animals that it had a marked effect on the virulence of several spirochetes, and that this effect was demonstrable only by the injection into animals of the organisms so treated. Thus, Swift and Ellis[13] showed that the serum of rabbits treated intravenously with salvarsan, and the serum of patients treated likewise with salvarsan or neosalvarsan exerted an inhibitory effect on the *Spirochaeta duttoni;* and that these spirochetes did not appear objectively (dark field) changed, but they would not cause infection in animals into which they were injected. These investigators also found that heating the serum at 56 C. (132.8 F.) for half an hour markedly increased the activity of the serum, and that this increase is due only in part to the destruction of some inhibiting sub-

6. Plaut: Deutsch. med. Wchnschr., 1910, xxxvi, 2237.
7. Meirowsky and Hartman: Med. Klin., 1910, vi, 1572.
8. Gibbs and Calthrop: British Med. Jour., 1911, i, 809.
9. Alsten, Henry: British Med. Jour., 1911, i, 360.
10. Swift and Ellis: New York Med. Jour., 1912, xcvi, 53-55.
11. Gonder, R.: Ztschr. f. Immunitätsforsch., Orig., 1912, xv, 57.
12. Castelli, G.: Ztschr. f. Chemotherap., Orig., 1912, i, 122 and 167.
13. Swift and Ellis: Jour. Exper. Med., 1913, xviii, 435.

stance. They also showed that the cerebrospinal fluid does not possess these inhibitory bodies.

The intraspinal injection of serum, taken an hour after the intravenous administration of salvarsan, was first employed by Swift and Ellis as a method of treating cerebrospinal syphilis. Their technic[14] was, in brief, the following: One hour after the intravenous injection of salvarsan 40 c.c. of blood are withdrawn directly into a bottle-shaped centrifuge tube, and allowed to coagulate, after which it is centrifugalized. The following day 12 c.c. of this serum are pipetted off and diluted with 18 c.c. of normal saline solution. This 40 per cent. solution is then heated at 56 C. for half an hour. By lumbar puncture the cerebrospinal fluid is withdrawn until the pressure is reduced to 30 mm. (water), after which enough of the serum solution is run in by gravity exactly to replace the cerebrospinal fluid withdrawn.

During the past year and a half the cases of cerebrospinal syphilis in the City Hospital at Bay View have been treated along the lines laid down by Ellis and Swift, with one important change—we injected undiluted serum in large quantities (25-35 c.c.). Thus, there was given, in many instances, twice as much actual serum at a single injection as was given by Ellis and Swift. The technic was the following: Salvarsan, 0.6 gm., was given intravenously, having been made up in water distilled within an hour of time of using, and the solution was injected at once. One hour later from 75 to 100 c.c. of blood were withdrawn into a large, sterile tube, and this was set aside to clot. After from one to eighteen hours the serum was pipetted off and centrifugalized at high speed for fifteen minutes in order to throw down any red blood-corpuscles present. The serum was then heated in a water-bath at 56 C. for half an hour and the intraspinal injection made at once. A lumbar puncture was done and the spinal fluid was slowly and carefully drained off into a graduated sterile cylinder. Not more than 35 c.c. were removed. The serum was then injected, a syringe and two-way stop-cock being used, the serum being drawn back into the syringe from the flask and then slowly injected through the needle. Great care was taken not to use any undue pressure in running in the serum. Enough was injected to replace exactly the amount of spinal fluid removed, whether this was only a few cubic centimeters or as much as 35 c.c. After the injection all pillows were removed, the foot of the bed elevated and the patient kept on his back in this position for at least an hour. The treatments were repeated at intervals of seven to twenty days, when possible, depending in part on the way in which the patient reacted.

The brief protocols of eight unselected cases are appended as illustrative of the method, with note of some of the difficulties and dangers,

14. Swift and Ellis: THE ARCHIVES INT. MED., 1913, xii, 331.

and the results achieved. Some cases are still under treatment at the time of this report.

REPORT OF CASES

CASE 1.—An unmarried colored laundress, aged 37, was admitted suffering from tertiary syphilis with cerebrospinal infection and syphilitic cerebral thrombosis.

History.—The primary infection occurred twenty years before onset of present illness. No secondaries showed but tertiary lesions appeared during the past five years. The patient had had slight, irregular mercurial treatment during past five years. Severe frontal headaches had existed for a week before admission, with the development of flaccid, left-side paralysis, which came on suddenly eighteen hours before admission with no loss of consciousness or convulsions.

Examination.—Complete left-side paralysis, which included the muscles of the face, arm and leg, with moderately exaggerated deep reflexes and positive Babinski's sign. Neck stiff and retracted. Several large, luetic ulcerations over lower abdomen.

Treatment and Result.—After admission the patient was given three injections of salvarsan and two intraspinal injections. In the first treatment blood was withdrawn seventy-two hours after the injection, and in the second twenty-four hours after injection. When discharged the patient was normal physically except for a slight residual paralysis of the flexors of the left forearm and a left pes equinus. On admission the cerebrospinal fluid showed twenty cells per cubic millimeter; when discharged there were seven cells per cubic millimeter. Wassermann was positive on admission, negative when patient was discharged.

CASE 2.—A married, white window-washer aged 44, was admitted ill with tabes dorsalis.

History.—Primary infection had occurred twenty years before admission. The present illness began five years before admission with sharp, shooting pains in the legs. Extreme ataxia had developed in past two years. The patient had had six weeks' treatment in a German army hospital at the time of primary chancre. About two months later he was given another short course of iodids and mercury.

Examination.—No sphincter disturbances. Argyll Robertson pupils present, knee-jerks absent, muscle sense in legs much impaired, marked ataxia, causing great difficulty in walking.

Treatment and Result.—Two intravenous injections of salvarsan and two intraspinal injections were given with consequent improvement. On admission the patient was very ataxic and suffering severely with lightning pains. After the second treatment the lightning pains had ceased entirely and he could walk much better. He felt so much improved that he refused to remain for further treatments.

CASE 3.—An unmarried white man, a clerk, aged 37, on admission was found to be afflicted with tabes dorsalis.

History.—The primary infection had occurred nine years prior to admission and seven years before onset of present illness, which was accompanied with lightning pains, gradually increasing unsteadiness in walking and dimness of vision. Bladder symptoms developed about a year after onset. At the time of the primary chancre the patient had local treatment and took pills by mouth for one month; during the past eighteen months, mercury and iodids continuously. One year ago he was given an intramuscular injection of salvarsan and a second three months later.

Examination.—Argyll Robertson pupils. General enlargement of lymph-nodes. Knee-jerks absent, muscle sense completely absent in the legs, moderate dulling of tactile and pain sense. Romberg sign markedly positive. Positive Wassermann in blood-serum and spinal fluid and ten cells per cubic millimeter in spinal fluid.

Treatment and Result.—Four intravenous injections of salvarsan, three of neosalvarsan, and six intraspinal injections were given. Total: salvarsan 2.7 gm., serum 116 c.c.; time, seventy-nine days. On admission the patient was unable to walk on account of the ataxia. At present he walks fairly well without assistance. He is now free from the lightning pains. The cells in the spinal fluid dropped from ten to six per cubic millimeter, and Wassermann reaction has changed from complete fixation to a very faint fixation. The reactions were very severe, prostrating the patient, and for this reason the treatments were discontinued.

Case 4.—An unmarried, white salesman, aged 37, was admitted with cerebrospinal syphilis and syphilitic lateral sclerosis.

History.—The primary infection was incurred fifteen years before onset of present illness two and a half years ago, when unsteadiness in walking developed, soon followed by weakness and stiffness of the legs. Paresthesias, numbness, girdle sensations and bladder symptoms developed within eighteen months. The patient has had several short mercurial courses by mouth and by inunction.

Examination.—General enlargement of lymph-nodes. Pupils unequal but react normally. Knee-jerks greatly exaggerated on both sides; ankle-clonus present and Babinski sign positive. Legs stiff and spastic and held in adduction. Patient walks with difficulty. Wassermann on blood-serum, 60 per cent fixation. Positive Wassermann and twenty-five cells per cubic millimeter in cerebrospinal fluid.

Treatment and Result.—Two intravenous injections of neosalvarsan, six of salvarsan and eight intraspinal injections were given; total: salvarsan, 3.9 gm., serum 178 c.c.; time, 195 days (two admissions). On admission the patient was so spastic as to be unable to stand on his feet and he was suffering severely with girdle sensations. These disappeared immediately and the spasticity became distinctly less marked until he can now walk without assistance and can go up and down stairs. In the spinal fluid the cells have fallen from twenty-five to five per cubic millimeter, and the Wassermann from complete fixation to very faint fixation.

Case 5.—A married white man, blacksmith, aged 45, was admitted with tabes dorsalis, partial optic atrophy and tertiary syphilis.

History.—Primary infection appeared as a chancre twenty-two years before admission. Present illness began four years before admission with dimness of vision, soon followed by lightning pains which have gradually become more and more intense. Unsteadiness in gait has developed during the past two years; no sphincter disturbances. At the time the chancre appeared a few local applications of calomel powder were made; no medicine was given internally. During the past four years the patient has had more or less continuous treatment with mercury and iodids.

Examination.—Moderate enlargement of lymph-nodes. Argyll Robertson pupils and primary optic atrophy. Knee-jerks absent; moderate ataxia of legs. Positive Wassermann and thirty-five cells per cubic millimeter in spinal fluid. Wassermann in blood-serum negative.

Treatment and Result.—Eight intravenous injections of salvarsan and eight intraspinal injections were given; total: salvarsan, 4 gm.; serum, 204 c.c.; time, 108 days. This patient had been unable to work for six months on account of the severe lightning pains. After the first treatment he was entirely free from pains except for one mild attack. The cell-count in the spinal fluid fell from thirty-five to five per cubic millimeter, and the Wassermann from positive to negative.

Case 6.—A white man, bookkeeper, widower, aged 54, was admitted suffering from tabes dorsalis.

History.—Primary infection occurred twenty-four years prior to admission. Initial lesion was not known, possibly a chancre. Two years before admission

the patient had an acute retention of urine, and examination at that time showed inactive pupils, knee-jerks faint, bladder atonic and anesthetic, and a positive Wassermann in spinal fluid. Eighteen months ago he began to suffer with severe pains in the back, coming on in paroxysms about every half hour, worse at night. A hemiplegia, three years ago has cleared up almost completely, the right side having been involved. The patient has had several short courses of iodids and mercury by mouth during the past two years.

Examination.—Argyll Robertson pupils. Unilateral sweating involving the whole left side of the head and neck. Slight residual weakness and exaggeration of reflexes of right arm and leg. Knee-jerk almost absent on left. Wassermann in blood-serum negative. Positive Wassermann and sixty-two cells per cubic millimeter in spinal fluid.

Treatment and Result.—Two intravenous injections of neosalvarsan, seven of salvarsan and nine intraspinal injections were given. Total: 3.9 gm., serum, 186 c.c.; time, seventy-seven days. When admitted the patient was almost prostrated on account of the severe and constant pain in the back. This steadily became less intense and the patient was quite comfortable when discharged. The cells in the spinal fluid fell from sixty-two to four per cubic millimeter and the Wassermann changed from positive to negative.

CASE 7.—An unmarried colored man, a cook, aged 45, was admitted under diagnosis of tabes dorsalis with optic atrophy.

History.—The primary infection had occurred twenty-seven years before onset of present illness, which began five years before admission, with unsteadiness and weakness of the legs; the subsequent history is typical. There has been incontinence of urine for three years. Lightning pains have been constant but not severe. Numerous gastric and laryngeal crises have occurred during the past year. The patient has had failing vision for eighteen months. For the past year the ataxia has been so severe that he has been practically helpless. The patient had a short course of local treatment at time of chancre; mercury and iodids by mouth during past year.

Examination.—Patient greatly emaciated. Complete loss of muscle sense in the arms and legs with exaggerated ataxia of the muscles of the face, arms and legs. Argyll Robertson pupils. Deep reflexes of arms and legs absent. Wassermann in blood-serum negative. Positive Wassermann and thirty-six cells per cubic millimeter in spinal fluid.

Treatment and Result.—Three intravenous injections of salvarsan and two intraspinal injections were given. Total: salvarsan, 1.2 gm.; serum, 35 c.c.; time, thirty-six days. Patient suffered an extreme reaction after the second intraspinal injection which was characterized by severe respiratory depression. Immediately after the injection, at 4 p. m., it was noticed that the patient seemed slightly dazed and did not answer questions so quickly as usual, although he was perfectly rational. Twenty minutes later the mouth began to twitch and pull to the right, the eyes turned to the right, and the right arm and leg began to jerk slightly. The respirations suddenly dropped to four a minute, and were very irregular, the sequence usually being two or three spasmodic respirations followed by a long period of apnea, sometimes lasting three-quarters of a minute. The jerking and twitching soon ceased and the patient became quite flaccid. At 6 p. m. it became necessary to give artificial respiration for about half an hour. A lumbar puncture was done at 7 p. m., and 10 c.c. of the spinal fluid, tinged with the serum, were allowed to drop out. At 8 p. m. another puncture was done and the spinal canal washed out three times with normal saline. It was noticed that the intraspinal pressure varied with the respirations in the normal manner, and there was no noticeable change in the condition of the patient when the canal was drained or filled with 35 c.c. of normal saline. The condition of the patient remained about the same until 1 a. m., when he suddenly sat up in bed, recognized those about him and talked rationally. In about fifteen min-

utes he again suddenly sank into apparent coma, with spasmodic and irregular respirations, four or five to the minute, and remained thus until 4 a. m., when, as before, he suddenly recovered, was perfectly rational and showed no signs of weakness. When questioned the patient asserted he was entirely conscious at all times and told with a fair degree of accuracy what was done and who was in the room. He suffered no pain, but simply could not breathe, although he felt a great desire to do so. The next day he was as well as usual. No more treatments were given and he left the hospital in a week, unimproved.

CASE 8.—An unmarried white man, a painter, aged 47, was admitted suffering from cerebrospinal syphilis.

History.—The primary infection occurred twenty-six years before admission. The patient was admitted on account of acute alcoholism. When examined the eyes showed Argyll Robertson pupils. For the past year he has suffered with pains in the back and giddiness. The patient had a short course of local treatment at time of chancre.

Examination.—Reflexes all normal, knee-jerks active. Wassermann on blood-serum negative. Faintly positive Wassermann and fifty-four cells per cubic millimeter in spinal fluid.

Treatment and Result.—Four intravenous injections of salvarsan and four intraspinal injections were given. Total: salvarsan, 2.4 gm.; serum, 118 c.c.; time, thirty-six days. The cells in the spinal fluid dropped from fifty-three to ten per cubic millimeter; the Wassermann remained positive. The patient felt perfectly well and could not be induced to remain longer in the hospital.

From the foregoing cases it is evident that the intraspinal method is a step forward in the treatment of tabes and cerebrospinal syphilis. The patients were uniformly relieved from pain, and as far as locomotion is concerned there was a slow but definite improvement, in some instances amounting almost to restitution to normal. It is in this respect that our cases seemed to do better than those of Swift and Ellis, perhaps on account of the larger doses of serum.

The psychic effect of the treatment is also marked. The patients became encouraged by the quick relief of pain and the first improvements in the control of motion and were faithful in their efforts to use the legs and practice walking, stair climbing, etc. The nutrition also rapidly improved under these stimuli. In fact, this very improvement was a hindrance in our series, for with the class of people under our care it was in most instances impossible to keep them in the hospital long enough to carry the treatment as far as we should have liked to do.

The effect of the treatment on the cerebrospinal fluid was very striking. The cell-count first fell to normal, the Wassermann reaction becoming negative some time after the cells were reduced. It has seemed from our observations that the Noguchi reaction was of little value during the carrying out of this treatment, since such large amounts of globulin were injected into the spinal canal. At any rate, the Noguchi reactions were positive, in most instances, after the cell-count and Wassermann indicated a normal fluid.

Where the patients are intelligent enough to appreciate the necessity for repetition of the treatment until the biologic indices of normal are

maintained, there need be but transient interruptions of regular occupation, lasting forty-eight to seventy-two hours as a rule. It is also true that while the risks are minimal, they do exist and in all probability very feeble, advanced tabetics or very acute cases of cerebrospinal lues should not be treated in this way.[15]

In those of our cases which were treated with the undiluted serum the reactions were sometimes more severe than those reported by Swift and Ellis—pain in the abdomen, legs and rectum, with occasional nausea and vomiting or dizziness, and in a few instances a little fever. We do not feel, however, that these constitute any contra-indication to the larger doses of whole serum, especially in the light of the apparently greater benefit to the patient. The reactions produced by the injection of the salvarsanized serum from another patient were very severe, as shown by two of our cases, the donors of the serum receiving an equal quantity of the same lot, at the same time and in the same way, having no reaction at all.

It is quite evident that so indirect a method of applying arsenic in the treatment of syphilis of the central nervous system cannot be considered as final; but we believe it is an advance on what has gone before and a spur to the findings of new preparations or new methods more suitable for the accomplishment of the desired results.

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15. One of us has treated by this method a tabetic with diabetes mellitus. The patient was told of the risk, but wanted to take any chance. He had had five previous intravenous treatments with salvarsan. Eighteen hours after the injection of 20 c.c. of salvarsanized serum he developed diabetic coma and was very ill for five days, when a large intravenous transfusion of sodium carbonate and glucose turned the scale and he recovered from the coma. He was not benefited by the treatment except in the disappearance of the lightning pains.

TWENTY-THREE CASES OF PNEUMONIA TREATED WITH ANTIPNEUMOCOCCUS SERUM *

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Through the cooperation of the Research Laboratory I was able to give a series of patients suffering from lobar pneumonia an antipneumococcus serum, and although the results were somewhat inconclusive, it seems desirable to publish them.

The series includes practically all of the pneumonia patients admitted to the service of the Second Medical Division of New York Hospital during the period of the test. This division receives all medical patients admitted to the hospital on the even days of the month, while those admitted on the odd days go to the first division. Although this method of assignment does not give a strict alternation of patients, yet it seemed justifiable to use the patients on the first division as controls, inasmuch as none of them received any serum.

The serum was taken from a horse immunized against many strains of pneumococci.[1] Usually it was injected directly into a vein of the arm at a temperature about that of the patient. This rule was departed from and the serum was given subcutaneously to two children on account of the difficulty of entering the vein with the needle, and also when second and later doses were given to four adult patients who had received the initial dose intravenously. The first doses for adults in the earlier cases was 100 c.c. This was increased later until, in one instance, an initial dose of 300 c.c. was given. Many of the patients received more than one dose so that the total amount of serum that an individual received ranged from 50 to 750 c.c. This maximal quantity was given in six doses spread over a period of five days.

A complete demonstration of the nature of the infecting organism was not essayed. Blood-cultures, however, were made in eleven cases. Only one of them (Case 11) showed the pneumococcus, the others being negative. In Case 6, pneumococci were cultivated from pus from the pleural cavity. Cultures were made from the sputum in several instances.[1] The diagnosis was based on the clinical evidences of pneumonia, special weight being given to the presence of rusty sputum and

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* From the New York Hospital and Research Laboratory, Department of Health, City of New York.

1. See Notes on Serum, Cultures, Etc., following.

the physical signs of a definite consolidation of the lung. In every case the subsequent course of the illness confirmed the diagnosis of lobar pneumonia and strengthened the probability of the existence of a pneumococcus infection.

The chief points of note in these cases are given in a very condensed form in Table 1, together with the details of the dosage of the serum. The time of onset of pneumonia is not uncommonly indefinite. Many patients give a history of an indefinite illness lasting for several days, during which time they may not have been confined to bed continuously. This may explain the duration of some of the more protracted cases of this series. Most of the patients, however, gave symptoms that fixed the beginning of the pneumonia with a reasonable degree of accuracy.

A review of the data of Table 1 shows that the series included twenty-three cases with sixteen recoveries and seven deaths, a mortality of 30.5 per cent. Of the seven patients who died, three were alcoholics, one of whom had suffered from two previous attacks of pneumonia, and all were very actively delirious. A fourth death was that of a baby (Case 4), who, in addition to pneumonia, had multiple abscesses of the scalp, double purulent otitis media, and empyema followed by pneumothorax. The remaining three deaths seemed to have been due to the frank intensity of the pneumonia.

Of the sixteen patients who recovered, eight had defervescence by crisis and eight by lysis. The crisis occurred on the following days: fourth, fifth to sixth, sixth, seventh, ninth, eleventh, twelfth, and on the eighteenth to nineteenth. The onset of the lysis in the other cases in which the patients recovered occurred on the following days: sixth, seventh (three cases), eighth, tenth and on the thirteenth day. In the remaining cases the onset of the lysis was scarcely definable because the fall of temperature was so gradual. Five patients had complications. In one instance this was serous pleurisy (Case 5). In cases 6 and 19 empyema developed. In Case 4 the signs and symptoms were those of empyema, but an exploratory puncture failed to find the pus. On the next day, however, there was evidence of pneumothorax, and on operation considerable pus was found. Two days later the child died with edema of the lungs. She was extremely ill from the time of admission and had in addition to the pleural complications, double otitis media for which the drum membranes were incised, and also many abscesses of the scalp. Otitis was detected in Case 18 also. The series offered an opportunity to observe the effects of the administration of large and frequent doses of horse-serum that had not been refined in any manner and that contained no preservative except a trace of chloroform.[1] Usually no immediate effects could be detected and the injection of the serum was

TABLE 1.—SUMMAR

Case No.	Sex,* Age in Years, Habits, Etc.	Condition of Patient				Blood-Culture, E
		Day of Admission	Invasion	Temperature Pulse Respiration	Site Lesion	
1	♂ 12	3	Chill	T. 104°–5° P. 132 R. 28	L. L.	Sterile
2	♀ 1¼	15 (?)	Chill	T. 104.8° P. 160 R. 50	R. U. L. U.	.....
3	♂ 41 Alcoholic	8	Chill	T. 103° P. 124 R. 48	R. M. R. L.	.....
4	♀ 5	6	.....	T. 106° P. 140 R. 48	R. U.	.....
5	♂ 26	2	Chill	T. 106° P. 116 R. 28	L. L.	Sterile
6	♂ 17	4	Chill	T. 105° P. 128 R. 40	R. L.	Sterile
7	♂ 30	5	Chilly	T. 103.6° P. 108 R. 32	R. U. R. M.	.....
8	♂ 30	2	Chill	T. 105° P. 112 R. 24	R. U.	Sterile
9	♀ 29	2	Cough, pain	T. 103° P. 140 R. 40	R. L.	Sterile
10	♂ 46	7	Chill	T. 104.6° P. 108 R. 32	L. L.	Sterile

* In this column ♂ denotes male and ♀ female.

Serum Administration			Outcome of Disease			
Day of Disease	Dose, Methods	Effect	Termination of Case	Serum Sickness and Day of Disease it Appeared	Complications	Remarks
4	50 c.c. intrav.	Chill. T. 3°+	Crisis 4th day. Recovery.	Purpuric rash, arthritis 8th day.	.....	Crisis a few hours after serum.
15	30 subc.	.....	Crisis 18th and 19th days. Recovery.	.....	.....	First and second injections without effect.
16	30 subc.					
17	30 subc.					
8	100 intrav.	T. 2°+	Resolution. Death 19th day. Temp. rose 4°; edema of linings.	.....	.....	Patient alcoholic; acute delirium; edema of lungs. Serum without effect.
9	100 intrav.					
10	100 intrav.					
11	100 intrav.					
6	30 subc.	T. 2°—	Death.	Erythema 19th day	Abscess of scalp, otitis media twenty-first day, pyopneumothorax, 600 c.c pus. Edema of lungs. twenty-fourth day.	Serum without any definite effect. Patient very ill on admission.
7	50 subc.	T. 3°—				
9	45 subc.					
11	50 subc.					
14	50 subc.					
3	100 intrav.	.....	Recovery.	Urticaria fever 17th day.	Pleurisy with effusion 150 c.c. aspirated.	No definite effect of serum. Temperature fell by lysis after last injection.
4	100 intrav.					
5	100 intrav.					
5	100 intrav.					
6	150 intrav.					
7	200 intrav.					
4	100 intrav.	T. 2°—	Recovery.	.....	Empyema. (Pneumococcus) operation.	Temperature fell gradually after first injection. No effect from later injections.
7	150 intrav.					
8	200 intrav.					
10	200 intrav.	T. 3°+				
6	200 intrav.	T. 3.5°—	Recovery.	Erythema 16th day.	.....	Sharp drop in T., P. and R. immediately after serum.
3	80 intrav.	Edema of lungs in one-half hour.	Crisis 9th day. Recovery.	26th day, T. 104°; 27th arthritis.	.....	Patient had symptoms of anaphylaxis. Serum stopped; no effect.
4	150 intrav.	.....	Recovery.	.....	.....	Resolution fourth week; no effect from serum.
6	175 intrav.					
9	200 intrav.	.....	Recovery.	.....	.....	Temperature fell by quick lysis after serum.

TABLE 1.—

Case No.	Sex,* Age in Years, Habits, Etc.	Condition of Patient				Blood-Culture, Etc.
		Day of Admission	Invasion	Temperature Pulse Respiration	Site Lesion	
11	♂ 39 Alcoholic	6	.....	T. 104° P. 100 R. 36	L. U. L. L.	Pneumo-coccus
12	♂ 16 In extremis	2	.....	T. 107°	R. L.	.....
13	♀ 2	5	.....	T. 105° P. 136 R. 40	L. L.	.....
14	♂ 38 Alcoholic; third attack	6	.....	T. 103.6° P. 124 R. 24	R. U.	.....
15	♂ 24	8 (?)	.....	T. 105.4° P. 102 R. 32	R. L.	Sterile Plasmodium malariae
16	♂ 24	3	Chill	T. 105° P. 144 R. 40	L. L.	Sterile
17	♂ 4½	11	Chill, cough	T. 101° P. 118 R. 32	L. L.	.....
18	♂ 30 Alcoholic	6	.....	T. 105° P. 128 R. 44	L. U.	.....
19	♂ 32	4	Chill	T. 104.6° P. 120 R. 44	L. L.	.....
20	♂ 13	3	Chill	T. 102° P. 120 R. 48	R. L.	Sterile
21	♂ 54	6	Chill	T. 104.8° P. 88 R. 24	R. U. R. L.	Sterile
22	♂ 20	5	Chill	T. 105.6° P. 120 R. 44	L. L.	.....
23	♂ 33 Alcoholic	4	.....	T. 102.4° P. 100 R. 48	R. L.	.....

—CONTINUED

Serum Administration			Outcome of Disease			
Day of Disease	Dose, Methods	Effect	Termination of Case	Serum Sickness and Day of Disease it Appeared	Complications	Remarks
6	200 intrav.	.....	Death.	.....	.....	No effect of serum on T., P. and R. Died three days later.
3	150 intrav.	.....	Death.	.....	.....	Died in eighteen hours.
8	100 intrav.	.....	Crisis 12th day. Recovery.	Erythema 14th day.	.....	No effect from serum. Crisis fourth day after serum.
6	150 intrav.	T. 2°—	Death.	.....	Edema of lungs	Temperature rose steadily; died eleventh day.
9 11	250 intrav. 50 subc.	T. 4½°—	Death.	.....	.....	Temperature fell after first injection, but rose again and rose after second injection.
4 5	250 intrav. 50 subc.	T. 3°—	Death.	.....	.....	Temperature dropped after first injection and then rose. No effect of serum.
12	50 subc.	T. 2°—	Lysis. Recovery.	.....	.....	Temperature fell 2° and then to normal after serum.
6 9	250 intrav. 50 subc.	.....	Lysis. Recovery.	13th day rash and fever; 16th arthritis.	.....	Slow lysis interrupted by serum sickness.
4 7	250 intrav. 50 subc.	.....	Lysis. Recovery.	Arthritis; fever 18th day.	Pleurisy with effusion; tenth day, 700 c.c. turbid serum; 12th, 350 c.c.; 25th, pus.	Serum without apparent effect.
4	250 intrav.	General pains.	Crisis 5th to 6th day. Recovery.	Urticaria after two hours.	.....	Crisis twenty-four hours after serum.
8	300 intrav.	.....	Lysis. Recovery.	.....	.....	Lysis rapid twenty-four hours after serum.
6	250 intrav.	.....	Crisis 6th to 7th day. Recovery.	.....	.....	Crisis nine hours after serum.
5	200 intrav.	Nausea, vomiting, pulse rapid and sweating.	Crisis 6th day. Recovery.	.....	.....	Crisis twenty-four hours after serum.

tolerated surprisingly well. In five patients, however, symptoms appeared that should be mentioned more in detail.

CASE 1.—A boy aged 12, received 50 c.c. of serum intravenously on the fourth day of his illness. A few minutes later the patient had a chill and his temperature rose from 104 to 107.2 F. This was followed by a startling drop, and within six hours of the injection his temperature was 97.6 F. The fever did not reappear, and convalescence was prompt and complete. I am inclined to regard this crisis as independent of the treatment, but at the time the sequence of events was very impressive.

CASE 8.—The patient was a negro, aged 30, a rather heavy drinker and a driver of horses. When 80 c.c. of serum had run into the vein, he suddenly developed severe dyspnea, a very rapid and weak heart-action, profuse sweating and edema of the lungs. The serum was at once stopped. Within a few minutes the symptoms began to abate and within half an hour the lungs were nearly clear and his condition was satisfactory. The subsequent course of the pneumonia was typical and but moderately severe. Six days later, on the ninth day of the disease, defervescence by crisis occurred. Convalescence was interrupted only by symptoms of serum sickness.

CASE 20.—A boy aged 13, during the introduction of the serum complained of headache, pain in the abdomen and later in the legs. The pulse was accelerating and the serum was stopped, but not until he had received 200 c.c. Two hours later a profuse urticarial rash appeared on the abdomen and lasted a few hours. The patient had no late symptoms of serum-sickness.

CASE 23.—A man, aged 33, a heavy drinker, when 150 c.c. of serum had been given, became nauseated and vomited. These symptoms ceased in about five minutes and 50 c.c. more of serum were given. At this time the pulse became rapid and the patient broke out in a profuse sweat. The serum was stopped and in a few minutes he was comfortable. He likewise had no late serum symptoms.

The effect of the serum on the leukocytes was determined by frequent blood-counts before and after the injections. No constant effects on the white cells could be made out. The results of some of these counts are detailed in Table 2.

Late symptoms due to the serum were noticed in eight patients. In consideration of the large doses of unrefined horse-serum, this is not a surprisingly large number. The symptoms were not usually severe.

During the time of this experiment the First Medical Division of New York Hospital treated fifteen cases of pneumonia with six deaths, a mortality of 40 per cent., as compared with 30.5 per cent. for the serum cases. Of their ten cases with recovery, in six, or 60 per cent., there was defervescence by crisis. In the serum cases 50 per cent. of the patients who recovered had crisis. Only two of the control cases had complications, a percentage of 13.3. In the serum cases five patients, 22 per cent., had complicating inflammations. It does not seem that these were due to the use of the serum, but the number of patients with inflammations of the pleura (four cases, 17.4 per cent.) is uncomfortably large.

TABLE 2.—Blood-Counts Before and After Injection

Case No.	Dose of Serum	Serum, c.c.	Leukocyte Counts					
			Before Serum		After Serum			
			No. of Cells	Percentage of Polynuclears	Hours After Serum	No. of Cells	Percentage of Polynuclears	Note
1	first	60*	25,000	86.5	2	19,000	85	
					4	30,000	87	} Crisis
					6	29,000	81	
					18	20,000	69.5	
					27	14,000	44.7	
2	first	30†	15,000	81.0	3	14,500	65	
					5	12,700	70	
					14	10,500	76	
	third	30†	13,000	70.0	2	12,900	70	
					13	11,000	70	
					36	15,900	71	
3	first	100*	50,000	94.5	2	49,500	95	
					4	50,000	91	
					16	55,000	97	
5	first	100*	24,400	85.0	2	26,900	92.5	
					4	25,000	86	
					6	25,400	89	
					8	25,900	88	
					10	24,500	88	
					21	20,000	85	
6	first	100*	27,800	88.5	2	29,000	91.5	
					4	26,900	94	
					6	28,800	93	
10	first	200*	20,800	93.5	2	29,000	94	
					4	27,000	93	
					6	23,000	90	
					18	21,100		
16	first	250*	14,000	86.0	2	16,400	92	
					4	15,400	89	
					6	12,200	84	

* Intravenous injection. † Subcutaneous injection.

A study of the results of this treatment leaves one doubtful of the influence of the serum, in modifying favorably the course of the pneumonia.

The prosecution of this experimental study necessitated much extra work in the wards and laboratory. This was performed by the members of my house staff, Drs. Howard, Kutil, Alling and Wildman. I wish to thank them for their enthusiastic willingness, which made these observations possible.

NOTES ON SERUM, CULTURES, ETC.

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The serum was prepared by injecting a horse with pneumococci from varied sources. These cultures were all typical inulin fermenting types. They were

isolated from the mouth in cases of Riggs' disease, from the sputum in cases of lobar pneumonia, from empyema, and from the meninges and blood of septic infections following the exanthems. The cultures used varied from time to time. As fresh cultures were isolated the older cultures were dropped. No attempt was made to raise the virulence of these cultures by passage through rabbits or mice. Although this may influence the protective value of the serum for these animals, it is open to question what influence such adaptation to a new host has on the curative value in man. The cultivation on artificial medium undoubtedly changes the pneumococci, but how far this influences their ability to stimulate curative substances in the horse is unknown. The purpose was to prepare an immune serum against the prevailing strains of pneumococci, using the strains as little removed from human sources as possible; and to test the value of such serum for general use, where the immunological tests on each case would be impossible.

The injections at first were cultures in broth to which was added horse-blood. Later the cultures were grown on agar and washed off with water. At first the increasing doses were of cultures which had been heated to 55 C. for one hour. When the dose had reached the maximum decided on, live cultures alternating with autolysate were used. The autolysate was prepared by allowing distilled-water suspensions of the cocci to stand in the ice-chest for one week. The live cultures or the autolysate were added to the heated vaccine, in increasing amounts, until only the unheated culture or autolysate was given.

The injections were given once a week subcutaneously. The maximum dose equaled the growth on agar in 10-pint Blake bottles. The horse was receiving the total amount of live and autolyzed cocci at the time it was supplied to Dr. Williams. The bleedings were made every two weeks. Every effort was made to supply the fresh serum to avoid any possibility of deterioration. After the serum was drawn from the clot, several cubic centimeters of chloroform were added to each liter. In bottling the serum for use, the supernatant serum was drawn off and the lower portion containing sediment and excess of chloroform discarded.

The material from the cases was examined both for diagnosis, and in the hope that the cultures isolated could be studied for their complement-fixing power, with the serum used, and that the protection value of the serum could be tested on the type of pneumococci found. This study was not made, partly because of other work, and partly because the strains were found to possess such low virulence for rabbits. When the intravenous inoculation of the growth from one blood agar slant was given, only two strains killed, one in forty-eight hours, the other after ten days. Cultures isolated from the heart's blood of these rabbits did not kill other rabbits when a somewhat smaller dose was used. The sputum from Cases 18 to 23, inclusive, was examined. They all showed an overwhelming preponderance of typical pneumococci. One case of the series showed the presence of an abundance of *Streptococcus mucosus*.

BOOK REVIEWS

MATERIA MEDICA, PHARMACOLOGY, THERAPEUTICS AND PRESCRIPTION WRITING FOR STUDENTS AND PRACTITIONERS. By Walter A. Bastedo, Ph.G., M.D., Associate in Pharmacology and Therapeutics at Columbia University. Cloth. Pp. 602. Illustrated. Philadelphia and London: W. B. Saunders Co., 1913.

The author believes that we are at the dawn of a new era of simple and practical therapeutics as the outcome of the adoption of laboratory methods in clinical research. For this reason he has given special prominence to the discussion of clinical studies on the action of drugs. It is this which gives the book its most striking character, namely, that it is right up to date. To the busy practitioner who has not had the time to keep up with the reading of the vast bulk of current literature this book ought to be most welcome. To the student this book offers, in a compact and most readable manner, a good practical course in these subjects; perhaps more practical and more useful than most of the other books on pharmacology and therapeutics at present in use.

It is perhaps somewhat unfortunate that the author has seen fit to use a therapeutic classification, for though it might be admitted that any classification in pharmacology is more or less unsatisfactory the therapeutic classification is the least systematic: for use that is made of drugs is after all more or less accidental rather than intrinsic. A certain amount of "lack-of-balance" in the treatment of the various topics becomes apparent, as, for instance, the large amount of space given to digitalis and the somewhat scanty discussion of some of the heavy metals. Under the heading of Cathartics a very brief discussion of the causes of constipation is introduced, which is probably not only unnecessary but even undesirable in a book of this character. Nevertheless Bastedo has given us a valuable text-book which it seems is destined to make a place of its own in this field.

PROTEIN SPLIT PRODUCTS IN RELATION TO IMMUNITY AND DISEASE. By Victor C. Vaughan, Victor C. Vaughan, Jr., and J. Walter Vaughan. Cloth; price, $3.00, one volume; total pp. 474. Philadelphia: Lea & Febiger, 1913.

The book describes the work of fifteen years along a well defined line of thought. Those who have followed the publications from Vaughan's laboratory during these years know what opposition many of his beliefs have encountered, so that it is with real enthusiasm that one reads the clear, simple statement here presented. For it is obvious that almost all his tenets have been amply upheld by recent workers in immunity.

The preface contains a general consideration of the chemical forces that work in the living cell, and the introduction deals with the subject of the protein structure of bacterial cells. It is pointed out that like all other proteins, bacterial proteins can be split by chemical agents or ferments into a very poisonous portion and a non-toxic residue. The remnant of the book is concerned with a discussion of these split products and their relation to sensitization, immunity and disease. The work advances in a most orderly and logical fashion. The toxic portion is first established as a true protein by an exhaustive series of chemical analyses, and then there follows a description of its preparation and physiological action. It is clear from these physiological tests that what Vaughan and his co-workers saw many years ago in the behavior of animals injected with his "poisonous portion" was the now well-known phenomenon of anaphylaxis. The identity of the author's "poisonous portion" and Friedberger's anaphylatoxin is discussed at great length and with many varying points of view throughout much of the rest of the book. The two substances seem to be identical.

Chapters 8, 9 and 10 deal respectively with tubercle bacillus protein, anthrax and pneumococci. Two important points are made in these chapters. It is shown that the poisonous group (toxophor) of the tubercle bacillus kills, as all other toxic groups do, with the symptoms of anaphylaxis, but has no sensitizing power. The non-toxic cell residue, however, can sensitize guinea-pigs to living tubercle bacilli. In experiments with anthrax and pneumococcus proof is advanced to support the hypothesis that pathogenicity does not depend on the amount of the toxic substance, but on the ability of micro-organisms to live and multiply in the body; that is to convert human proteins into bacterial proteins.

In Chapter 11, which occupies about one-quarter of the book, the whole subject of anaphylaxis and protein sensitization is discussed. The argument, following a short historical sketch of the knowledge of anaphylaxis, is in presentation of the author's theory of the mechanism of anaphylaxis. Contrary to the generally accepted view that a mixed protein like blood-serum contains one protein which sensitizes and another which is toxic, he believes that the sensitizing and toxic proteins are groups in the same molecule. The process of sensitization confers the power on certain body cells of splitting such a protein molecule and liberating the toxic portion.

The chapter on protein fevers is most interesting and the protocols and charts quite conclusive. The last chapter, dealing with the phenomenon of infection, is likewise most suggestive and instructive. Here, as elsewhere in the book, it is strongly urged that vaccine therapy has great dangers as well as power to help. Consequently the author seeks the soluble protein sensitizer free from its toxic group. The book is so full of ideas and of sound experimental observation that a full discussion would require much more space. It is an exceptionally good piece of work, suggestive and stimulating, and the record of a most thoughtful and purposeful effort to demonstrate the obscure relationships of the protein poisons and infectious diseases.

A CORRECTION

NEW YORK, May 25, 1914.

To the Editor, Archives of Internal Medicine:—In the article printed in your valuable journal for March, 1914, on Ulcus Ventriculi, by myself and Dr. I. W. Held, I find the quotation from the article of J. Kauffman entitled, "Mangel an Magen-Schleim," from the *Archiv für Verdauungs-Krankheiten*, 1907, p. 616, to be incorrect. The first sentence reads that in hyperacidity the mucus in the stomach is increased. J. Kauffman found that in cases in which there is a diminution in the normal mucus of the stomach there is pain simulating hyperacidity, although the acidity is normal. On that he bases his assertion that silver nitrate is beneficial in cases of irritation of the stomach in which the mucus is diminished.

M. H. GROSS.

40 East Forty-First Street.

A CORRECTION

Dr. Edward Perkins Carter wishes to make correction in the figures as they appeared in the manuscript of his paper on "Clinical Observations on Defective Conduction in the Branches of the Auriculoventricular Bundle," in the May, 1914, number of the ARCHIVES, at page 801, as follows: Page 809, line 26, for "0.13 and 0.18" read 0.12 and 0.20; p. 809, line 27, for "0.18" read 0.20.

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